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Abstract

Serotonergic Drugs, Their Postmortem Distribution in Man, and Their Effect on Serum Serotonin Levels

by Kabrena E. Goeringer

Chairperson of the Supervisory Committee
Professor Gary D. Christian
Department of Chemistry

The discovery of multiple serotonin receptor subtypes has encouraged the development of drugs which possess more specificity of action than their earlier-developed counterparts. These drugs have been involved in an increasing number of fatalities in the State of Washington since 1995, which is an unexpected development considering their smaller side effect profile compared to that of older drugs. This thesis describes experiments on a range of drugs with serotonergic activity in an attempt to understand circumstances surrounding adverse drug reactions associated with them.

Analytical methods using gas chromatography/mass spectrometry and high-performance liquid chromatography with photodiode array detection were first developed for tramadol, a centrally acting analgesic with serotonin reuptake inhibition ability, and its metabolites in postmortem blood. Derivatization was not required to isolate any of the compounds. These methods were applied to cases of suspected drug-related deaths and drug-impaired driving.

Methods were next developed using GC/MS and HPLC/PDA to separate several atypical antidepressants and their active

metabolites. These methods were used to analyze blood from suspected drug-overdose death cases. In eight cases, other tissue samples (liver, bile, urine, vitreous fluid, and gastric contents) were analyzed to study postmortem distribution of these drugs.

Finally, a method reported in the literature for determination of serotonin and its metabolites in human platelet-poor plasma using HPLC with electrochemical detection was evaluated for use in postmortem cases. The method was applied to 19 cases of either no drug present or clear Selective Serotonin Reuptake Inhibitor overdose. Stability of serotonin in plasma was also investigated.

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# Serotonergic Drugs, Their Postmortem Distribution in Man, and Their Effect on Serum Serotonin Levels

by

Kabrena E. Goeringer

A thesis submitted in partial fulfillment
of the requirements for the degree of

Master of Science

University of Washington

1996

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Abstract

Serotonergic Drugs, Their Postmortem Distribution in Man, and Their Effect on Serum Serotonin Levels

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The discovery of multiple serotonin receptor subtypes has led to the development of new drugs, especially antidepressants, which possess more specificity of action than their earlier-developed counterparts. These drugs have been involved in an increasing number of fatalities in the State of Washington since 1995, which is an unexpected development considering their smaller side effect profile compared to that of older drugs. This thesis describes a series of experiments on a range of drugs with serotonergic activity in an attempt to understand circumstances surrounding adverse drug reactions associated with them.

Analytical methods using gas chromatography/mass spectrometry (GC/MS) and high-performance liquid chromatography (HPLC) with photodiode array detection (PDA) were first developed for tramadol, a centrally acting analgesic with serotonin reuptake inhibition ability, and its metabolites, *n*- and *o*-desmethyltramadol, in postmortem blood. Derivatization was not required to isolate any of the three compounds. These methods were then applied to cases of suspected drug-related deaths and drug-impaired driving.

Methods were next developed using GC/MS and HPLC/PDA to separate a range of atypical antidepressants and their active metabolites. As with tramadol, these methods were used to analyze blood from cases of suspected drug-overdose deaths. In eight of the cases, other tissue samples (liver, bile, urine, vitreous fluid, and gastric contents) were obtained and used to study postmortem distribution of these drugs.

Finally, a method reported in the literature for determination of serotonin and its metabolites in human platelet-poor plasma using HPLC with electrochemical detection was evaluated for use in postmortem cases. The method was applied to 10 cases where no drug was present and 9 cases of clear overdose involving Selective Serotonin Reuptake Inhibitors. Stability of serotonin in plasma was also investigated.

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"Part of what makes people uneasy about Prozac is precisely that it works so well and has so few side effects. Prozac is enormously seductive ... This seduction is legitimately worriesome because we know that some drugs, especially those taken chronically, will have unknown or even late-appearing side effects ... Concern over unforseen or tardive effects is realistic, because Prozac has been around too briefly for anyone to know its long-term effects."

Peter D. Kramer, M.D., Listening to Prozac

# CHAPTER 1: GENERAL INTRODUCTION

## 1.1. Serotonin.

Serotonin, or 5-hydroxytryptamine (abbreviated as 5-HT), is a circulating monoamine implicated in the regulation of numerous physiological processes throughout the body. The exact sites and modes of action of 5-HT are not well understood, due mainly to the large number of 5-HT receptor subtypes -- fifteen, to date -- which have been identified and confirmed through cDNA cloning (1). It has been recognized that a wide range of physiological problems might be able to be treated through the development of drugs which specifically target one or more of these receptor subtypes. The location, type of signal transduction, and function of each receptor subtype is shown in Table I.1.

Table I.1. Serotonin Receptor Subtypes. (Taken from Gilbert & Goodman's *The Pharmacological Basis of Therapeutics*)(1).

| Subtype | Signal Transduction | Location | Function |
|---|---|---|---|
| $5\text{-HT}_{1a}$ | inhibition of AC | raphe nuclei, hippocampus | autoreceptor |
| $5\text{-HT}_{1b}^{*}$ | inhibition of AC | subiculum, substantia nigra | autoreceptor |
| $5\text{-HT}_{1d}$ | inhibition of AC | cranial blood vessels | vasoconstriction |
| $5\text{-HT}_{1e}$ | inhibition of AC | cortex, striatum | -- |
| $5\text{-HT}_{1f}^{\dagger}$ | inhibition of AC | brain & periphery | -- |

Table I.1 (Continued).

| Subtype | Signal Transduction | Location | Function |
|---|---|---|---|
| $5\text{-HT}_{2a}$ D receptor | activation of PLC | platelets, smooth muscle, Cerebral cortex | platelet aggregation contraction neuronal excitation |
| $5\text{-HT}_{2b}$ | activation of PLC | stomach fundus | contraction |
| $5\text{-HT}_{2c}$ | activation of PLC | choroid plexus | -- |
| $5\text{-HT}_{3}$ M receptor | ligand-operated ion channel | peripheral nerves | neuronal excitation |
| $5\text{-HT}_{4}$ | activation of AC | hippocampus | neuronal excitation |
| $5\text{-HT}_{5a}$ | unknown | gastrointestinal tract | unknown |
| $5\text{-HT}_{5b}$ | unknown | hippocampus | unknown |
| $5\text{-HT}_{6}$ | activation of AC | striatum | unknown |
| $5\text{-HT}_{7}$ | activation of AC | hypothalamus, intestine | unknown |

Note: AC: adenyl cyclase; PLC: phospholipase C
* Also referred to as $5\text{-HT}_{1D\beta}$
• Also referred to as $5\text{-HT}_{1E\beta}$

By designing drugs which act with more specificity, it is hoped that the likelihood of untoward side-effects will be diminished. The Selective Serotonin Reuptake Inhibitors (SSRI's) are a class of antidepressants with such specificity of action. Because these drugs target only 5-HT receptors, the number of side effects which

occur are much less than those associated with the use of tricyclic antidepressants, which also target norepinephrine and dopamine.

### 1.1.1. Sites of 5-HT Action.

Serotonin is not an endogenous substance; it is synthesized from the L-tryptophan in a variety of foods, such as turkey and Chianti, via the sequence shown in Figure I.1. After metabolism by

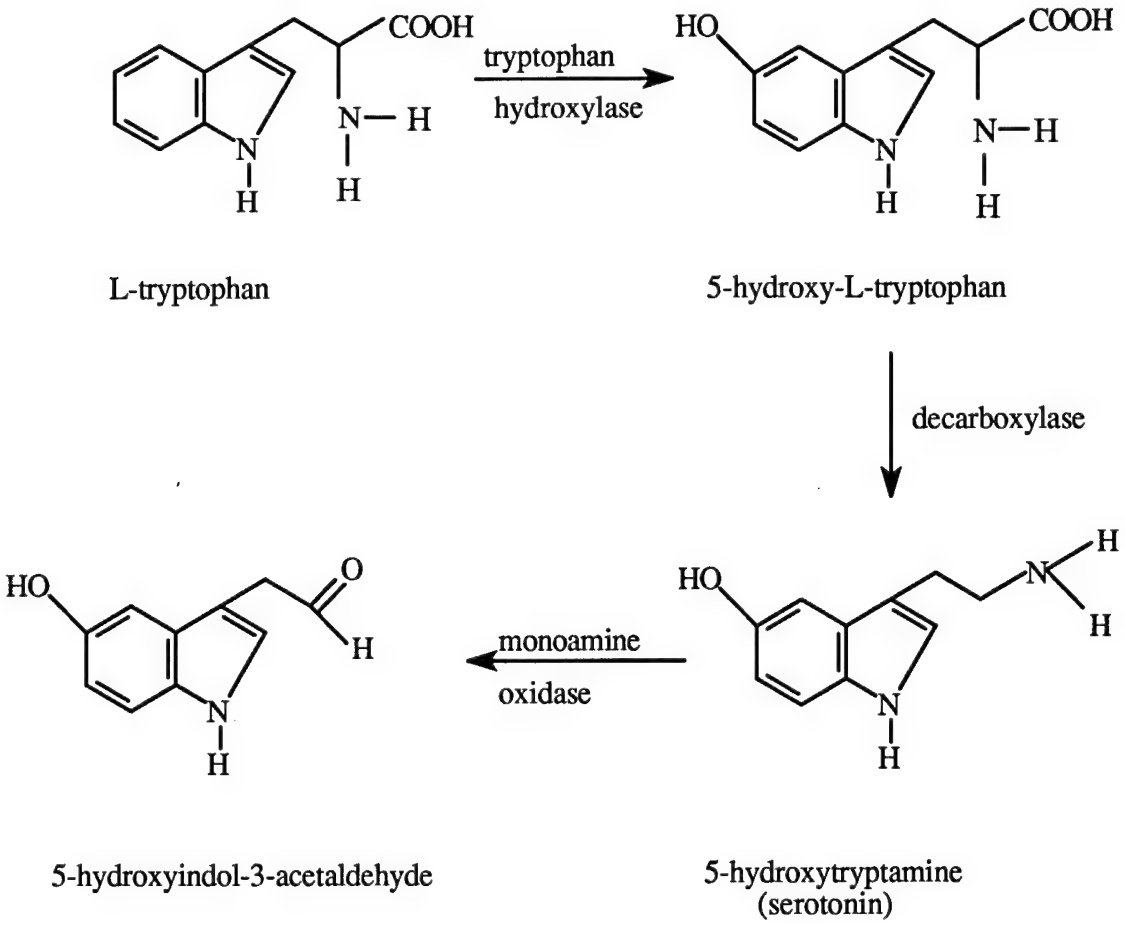



Figure I.1. Synthesis and metabolism of serotonin.

monoamine oxidase-A (MAO-A) to 5-hydroxyindol-3-acetaldehyde, it is quickly broken down further to 5-

hydroxyindole-3-acetic acid (5HIAA) and 5-hydroxytryptophol by aldehyde dehydrogenase and aldehyde reductase, respectively. The oxidation to HIAA is by far the major metabolic pathway (1). The areas of storage, secretion, and action follow.

**1.1.1.1. Enterochromaffin Cells and Gastrointestinal Tract.** Although 5-HT is primarily stored and secreted by platelets and enterochromaffin cells in the gastrointestinal mucosa, it also acts as a neurotransmitter in the central nervous system. This monoamine's physiological role differs in different areas of the body. For example, it plays a major role in regulating gastrointestinal motility. The enterochromaffin cells are the source of circulating 5-HT, which enters the portal vein and is subsequently metabolized by MAO-A in the liver (2).

**1.1.1.2. Platelets.**

Another principal storage site for 5-HT is platelets, although the monoamine is not synthesized there. It is taken up from the circulation and stored in secretory granules by active transport; $Na^+$-dependent transport across the surface membrane of platelets is followed by uptake into dense core granules via an electrochemical gradient generated by a $H^+$-translocating ATPase (3).

The main role of platelets is to plug holes in injured endothelial cells. Under normal vascular conditions, platelets release 5-HT to promote vasodilation and platelet aggregation, which tends to seal vascular holes and tears without causing thrombus formation (4).

However, in abnormal conditions, such as endothelial damage due to peripheral vascular disease, vasoconstriction can occur when 5-HT levels are increased (5). In a study on the status of 5-HT in whole blood and plasma (6), the authors found differences between men and women in plasma and whole blood 5-HT (both higher in women) and in plasma 5HIAA (lower in women). They concluded these differences may reflect a differential whole body 5-HT function between the sexes.

**1.1.1.3. Cardiovascular System.**

5-HT induces a variety of responses by the heart which result from activation of various 5-HT receptor subtypes. 5-HT has chronotropic and inotropic effects, and it increases the vasoconstriction produced by noradrenaline, angiotensin II, and histamine, which reinforce the hemostatic response to 5-HT (3, 7).

**1.1.1.4. Brain.**

Finally, a multitude of brain functions are influenced by 5-HT, including sleep, cognition, sensory perception, motor activity, temperature regulation, nociception, appetite, sexual behavior, and hormone secretion. Fourteen of the fifteen known 5-HT receptors are expressed in the brain, often in overlapping areas. The authors of one study, in which levels of the neurotransmitters norepinephrine, epinephrine, dopamine, and 5-HT were measured in 49 regions of the brain (8), found that the highest 5-HT concentrations were in the frontal and parietal lobes; specifically,

in the hypothalamus, the amygdala, the substantia nigra, nucleus ruber, oliva inferior, locus coeruleus and raphe nuclei.

### 1.1.2. Effects of Fluctuation of 5-HT in Tissues.

Low levels of 5-HT are commonly associated with clinical depression. Multiple postmortem studies have been performed which compared the brains of otherwise physically healthy people who were found to have committed suicide to those who died as the result of an accident. What distinguished the suicides was low brain 5-HT levels (9). In cases of depressive illness, serotonergic drugs are often given for treatment, with varying degrees of success.

By way of contrast, high brain 5-HT levels are associated with dominance. In studies of dominance hierarchy in multimale, mixed-sex troops of captive vervet monkeys, each troop was found to have one male monkey in whose bloodstream there was a distinctly elevated 5-HT level (1 1/2 times higher than that of other males in the troop), and in every instance, this was the dominant male (10). If the dominant male was removed, his 5-HT level fell almost 50 percent, below that of an average subordinate male, whereas that of newly dominant monkeys rose almost 60 percent. Further, if the original leader was then returned to the troop within ten weeks, in every case he would resume the dominant role and his 5-HT levels would return to normal, but that of the interim dominant male would fall below his original level.

A consequence of extremely elevated 5-HT concentrations is a condition of serotonergic hyperstimulation known as the "serotonin syndrome", which is distinguished by one or more of the following symptoms: mental status changes including confusion and hypomania, restlessness, myoclonus, hyperreflexia, diaphoresis, shivering, tremor, diarrhea, and incoordination (11) (see Table I.2).

Table I.2. Most Commonly Reported Symptoms of Neuroleptic Malignant Syndrome (NMS) & Serotonin Syndrome (SS).

| Symptom | NMS | SS |
|---|---|---|
| mental status changes | X | X |
| restlessness | X | X |
| myoclonus | | X |
| hyperreflexia | | X |
| diaphoresis | X | X |
| shivering | X | X |
| hyperthermia | X* | X† |
| tremor | X | X |
| diarrhea | | X |
| incoordination | X | X |
| autonomic dysfunction | X | |

Table I.2 (Continued).

| Symptom | NMS | SS |
|---|---|---|
| rigidity | X | |
| muteness | X | |

* Body temperature range 39-42° C
♦ Body temperature = 38°C

These symptoms bear strong resemblance to neuroleptic malignant syndrome (NMS), which involves suppression of spontaneous movements and complex behaviors while leaving spinal reflexes and unconditioned nociceptive-avoidance behaviors intact (9). However, differential diagnosis can be made by noting the presence or absence of benzodiazepines, and the presentation of restlessness and/or myoclonus, both of which are contra-indicative of NMS. It has been theorized that serotonin syndrome and NMS may be two forms of a more generalized hyperthermic syndrome (12).

## 1.2. Serotonergic Drugs.

A number of drugs are currently available which have serotonin reuptake inhibition ability as part of their mode of action. Characteristics of these drugs are outlined below.

### 1.2.1. Tricyclic Antidepressants.

Tricyclic antidepressants (TCA's) are commonly called norepinephrine reuptake inhibitors, but they affect several other sites in addition. As such, many people refer to them as "dirty"

(pharmacologically nonselective). The biochemical activity of these drugs is shown in Table I.3. Other limitations of these standard antidepressants include a significant lack of response in up to 20-30% of patients, lag time to response (usually 2-4 weeks), and common adverse effects (anticholinergic and cardiovascular side effects) (13). However, standard tricyclics have half-lives of approximately one day, which enhances compliance and takes advantage of the sedative effects of these drugs (13).

Table I.3. Biochemical Activity of Tricyclic Antidepressants.

| | Reuptake Inhibition | | | Receptor Affinity | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Drug name | ST | DA | NE | $\alpha_1$ | $\alpha_2$ | $H_1$ | musc | $D_2$ | mu-opiod |
| amitriptyline | ++ | 0 | ± | +++ | ± | +++ | ++++ | ± | 0 |
| imipramine | + | 0 | + | + | 0 | + | ++ | ± | 0 |
| amoxapine | 0 | + | ++ | ++ | 0 | + | ± | ++ | 0 |
| desipramine | 0 | 0 | +++ | + | 0 | 0 | + | 0 | 0 |
| maprotiline | 0 | 0 | ++ | + | 0 | ++ | + | + | 0 |

Abbreviations and symbols: NE = norepinephrine; DA = dopamine; ST = serotonin; + to ++ = active to strongly active; ± = weakly active; 0 = lacking

The apparent volume of distribution ($V_d$) is the extent of distribution of drug at equilibrium, and relates amount of drug in the body (A) to the plasma drug concentration (C) by the equation V = A/C. For tricyclics, this value is high, between 15-20 l/kg.

Consequently, these drugs are subject to postmortem redistribution.

In many cases, the major metabolites for tricyclics are pharmacologically active. Most TCA's, including amitriptyline and nortriptyline, are known substrates for the cytochrome P-450 isoenzyme 2D6 (CYP2D6), and as such are subject to increased half-lives and peak concentrations due to saturation of this isoenzyme in the presence of other 2D6 substrates and/or inhibitors. There are no known inducers of 2D6.

### 1.2.2. Selective Serotonin Reuptake Inhibitors (SSRI's).

Drugs which are classified as Selective Serotonin Reuptake Inhibitors (SSRI's) differ from the tricyclics in that even their active metabolites are selective for serotonin reuptake inhibition. In addition to depressed patients, SSRI's have been used successfully to treat such wide-ranging afflictions as bulimia nervosa, obsessive-compulsive disorder, anxiety, and migraines with few side effects due to the specificity of their pharmacological effects.

The specific mechanism of SSRI's is to occupy central 5-$HT_2$ receptors, where they act as monoaminergic antagonists (13). This is believed to lead to brainstem and spinal cord activation of the 5-$HT_{1a}$ receptor, which has been implicated in serotonin syndrome. The blockade of amine uptake is immediate, although the antidepressant effects can take up to several weeks to appear. Fluoxetine (Prozac) is perhaps the most common SSRI in use, but

sertraline (Zoloft), paroxetine (Paxil), and venlafaxine (Effexor) are also becoming more common. The biochemical activity of these drugs is shown in Table I.4.

The principal advantages of SSRI's over TCA's are that they cause minimal to no cardiotoxicity, anticholinergic effects, sedation, or weight gain, and they are relatively safe in overdose. It is worth noting, however, that little is known about the long-term side effects of these drugs. With the exception of paroxetine, the SSRI's all have active metabolites. The half-lives of these drugs and their metabolites range from a few hours to several days. The longer half-lives are potentiated by the fact that most SSRI's and some of their active metabolites exhibit autoinhibition (inhibition of a drug's metabolism by itself) (14). This gives rise to a non-linear

Table I.4. Biochemical Activity of SSRI's.

| | Reuptake Inhibition | | | Receptor Affinity | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Drug name | ST | DA | NE | $\alpha_1$ | $\alpha_2$ | $H_1$ | musc | $D_2$ | mu-opiod |
| fluoxetine | +++ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| fluvoxamine | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| paroxetine | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| sertraline | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| venlafaxine | ++ | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 |

Abbreviations and symbols: NE = norepinephrine; DA = dopamine; ST = serotonin; + to ++ = active to strongly active; ± = weakly active; 0 = lacking

relationship between dose and concentration and an increased half-life with increased dose. Fluoxetine, norfluoxetine, fluvoxamine, and paroxetine all exhibit autoinhibition.

As with the TCA's, many SSRI's are substrates for CYP2D6, and are hence subject to decreased clearance due to inhibition of 2D6 when other substrates and/or inhibitors of this isoenzyme are also present.

### 1.2.3. Atypical Antidepressants.

Atypical antidepressants such as bupropion do not fit neatly into either of the two earlier-mentioned categories. Bupropion is a very weak inhibitor of the neuronal reuptake of norepinephrine, dopamine, and 5-HT, but it preferentially inhibits dopamine reuptake (1). However, it may be converted *in vivo* to active metabolites with amphetamine-like activity, inhibiting both dopamine and norepinephrine reuptake. It is thought to lead to slight elevations in blood pressure, and may down-regulate ß-receptors and potentiate dopamine activity postsynaptically.

Trazodone and its newer congener, nefazodone, are atypical antidepressants with some serotonergic activity, although less than the SSRI's. The active metabolite of both trazodone and nefazodone, *m*-chlorophenylpiperazine (mCPP), also have activity as agonists of 5-$HT_1$ receptors and may indirectly facilitate noradrenergic transmission. As with many SSRI's nefazodone exhibits autoinhibition.

The half-life of bupropion is approximately 12 hours, while that for trazodone is around 6.5 hours. Although trazodone does not appear to be subject to postmortem redistribution due to its small volume of distribution (~1.0 l/kg), bupropion does, with a volume of distribution of approximately 7 l/kg.

### 1.2.4. Tramadol (Ultram, Ortho-McNeil).

Tramadol is a centrally acting, binary analgesic which is neither an opiate-derived nor a nonsteroidal anti-inflammatory drug (NSAID). It undergoes *n*- and *o*-demethylation to *n*-desmethyltramadol and *o*-desmethyltramadol, and is a racemic drug believed to possess two modes of action. The (+)-enantiomer binds weakly to the mu-opioid receptor (6000-fold less than morphine), which is the basis for the drug's claimed low tolerance and dependence potential for treatment up to six months (15). Used in therapy as a racemic mixture, the (+)-enantiomer weakly binds to the mu-opiod receptor, and both enantiomers inhibit serotonin and norepinephrine reuptake.

Tramadol's major active metabolite, *o*-desmethyltramadol, shows higher affinity for the mu-opiod receptor and has twice the analgesic potency of the parent drug. The synergism of these effects contributes to T's analgesic properties, with the (+)-enantiomer exhibiting a 10-fold higher analgesic activity than the (-)-enantiomer.

Tramadol is used to control moderate pain in several chronic pain settings, including osteoarthritis and post-operative cases.

Although initially thought to exhibit low abuse potential, Ortho-McNeil, the manufacturer of the drug, recently reported a large number of adverse events attributed to tramadol involving drug abuse by opioid-dependent patients, allergic reactions, and seizures. The high number of adverse reactions has prompted the company to update the prescribing information for the drug.

As with most of the other serotonergic drugs mentioned thus far, tramadol is a substrate for 2D6, and thus may be subject to metabolic inhibition in cases where other 2D6 substrates are detected. Its half-life is approximately 6 hours, and its volume of distribution is 2.7 L/kg.

**1.3. Postmortem Distribution and Redistribution.**

Postmortem distribution is a phenomenon first observed in a number of reports (16-20) which suggested that drug concentrations in blood specimens from various areas of the body may change during the postmortem interval prior to autopsy. In a study of this subject, Prouty and Anderson (21) discuss several reports in which the central blood concentration of a particular drug was significantly higher than that of peripheral blood. They analyzed specimens from multiple sites and concluded that no one collection site consistently produced the highest concentration and that it is impossible to predict which specimen will exhibit the largest change in drug concentration over time.

The first study which indicated postmortem redistribution may occur with TCA concentrations was performed by Bandt in 1980

(16). In this study, blood samples taken from a number of sites as well as serially from the same site were analyzed. Bandt concluded that TCA concentration generally increases with time and that the drug concentration is a function of the origin of the blood specimen. Bandt suggested that the process of postmortem redistribution may be driven by diffusion via a concentration gradient in which drugs are released from the liver and drug-rich liver blood, then diffuse into the larger vessels and subsequently into the right atrium. Drugs with high volumes of distribution (greater than 3 L/kg) are hence more prone to postmortem distribution, as are drugs which are highly tissue-bound.

### 1.4. Toxicity and Interpretation.

The therapeutic and toxic ranges for the drugs investigated in this research are shown in Table I.5. The CYP450 isoenzyme for which each drug is a substrate (if known) is also listed. Interpretation of toxicological results in cases where serotonergic drugs are found is often difficult because of the almost universal presence of other drugs. In many cases, the concentrations of drugs present are within therapeutic ranges. Therefore, other mechanisms of toxicity must be at work.

As was already mentioned, many serotonergic drugs are substrates for CYP2D6. Because such drugs are often found in combination, enhanced pharmacological effect due to longer half-lives and increased peak plasma concentrations of the parent drug

may result in toxic side effects which play a significant role in these fatalities.

Table I.5. Pharmacological Data for Drugs Investigated.

| Drug | Therapeutic conc, mg/L* | Toxic conc, mg/L* |
|---|---|---|
| amitriptyline (2D6) | 0.06-0.22 | >1.00 |
| nortriptyline (2D6) | 0.01-0.375 | >1.00 |
| doxepin | 0.03-0.15 | >0.10 |
| trazodone (3A4)† | 0.49-2.30 | >15.00 |
| bupropion‡ | 0.01-0.39 | 7.30 |
| fluoxetine (2D6, 2C19) | 0.06-0.453 | 1.30-6.80 |
| fluvoxamine (inhibits 1A2, 2D6, 3A4) | 0.02-0.42 | > 0.42 |
| sertraline (2D6, 3A4) | 0.03-0.19 | >0.61 |
| paroxetine (2D6, inhibits 2D6, 3A4) | 0.031-0.062 | ≥0.24 |
| venlafaxine (2D6, 3A4) | 0.07 | >0.245 |

* Levels are taken from Baselt (22) except fluvoxamine, which came from the USPDI Update (23).
† Trazodone's congener, nefazodone, has been shown to be a substrate for CYP3A4, so trazodone is likely also a substrate for this isoenzyme (4).
‡Bupropion's morpholinol metabolite is a suspected CYP2D6 substrate (116).

The serotonergic activity of these drugs must also be kept in mind when interpreting toxicological data. Even in cases where the drugs present are within therapeutic ranges, the synergistic effect of several serotonergic drugs could contribute substantially to toxicity. The autoinhibition ability of many of the SSRI's may be a factor in such cases.

Lastly, in subjects with heart disease, increased serum serotonin levels may contribute to toxicity. As mentioned earlier, the contracting action caused by 5-HT may cause vasoconstriction. The combination of increased serum serotonin levels due to the presence of serotonergic drugs and the decreased ability of the endothelium to metabolize 5-HT due to ischemic damage compounds the seemingly contradictory vasoconstriction seen with a damaged endothelium (25).

### 1.5. Analytical Methods for Serotonergic Drugs.

Due to the growing popularity of these drugs, extensive research has been completed with regard to their pharmacokinetics (26, 27) pharmacological effects (13, 28) and routes of metabolism (29, 30) as well as therapeutic drug monitoring (31, 32). The majority of these studies involve the development of high performance liquid chromatography (HPLC) and/or GC isolation methods to enable the monitoring of specific drug levels in a patient. Others, which involve development of isolation methods to gain an understanding of the pharmacokinetics and pharmacological effects of SSRI's, or to elucidate the metabolic pathways of these drugs, also focus on

detecting the drug at therapeutic levels in the patient. All of these either involve isolation of the drug from only one or two types of biological matrix or focus on only one or two SSRI's. None of these articles, however, provide a comprehensive toxicological tool for helping to determine cause of death.

A few articles focus on the toxicology of monoamine oxidase-inhibitors (MAO-I's) (33) and tricyclic antidepressants (12), and one discusses the development of isolation methods for sertraline and its major metabolite in postmortem specimens, as well as discusses the postmortem distribution of the drug (34). However, none of these studies compare postmortem distribution over the range of drugs with serotonergic activity. In addition, it should be noted that there is a general lack of information in the literature about levels of most of these drugs resulting from prolonged chronic therapy.

Because all of the serotonergic drugs are basic drugs, the liquid/liquid extraction procedure for basic drugs used at the Washington State Toxicology Laboratory, where the present research was conducted, can be used for all drugs and metabolites studied in this research. In this method, basic drugs are extracted into *n*-butyl chloride, back-extracted into 3M hydrochloric acid, and then re-extracted into chloroform for analysis. Although the metabolites are generally more polar than the parent drugs, they are still extractable may be analyzed without derivatization. Any quantitative losses due to analysis become unimportant if the

analyst spikes a set of blood samples with calibration standards, and extracts and analyzes them with the unknowns.

Serotonergic drugs are amenable to gas chromatography with nitrogen-phosphorous detection (GC/NPD) due to their amine content, assuming a column with sufficiently low polarity to elute the metabolites is used. The universality of gas chromatography with mass spectrometry (GC/MS) makes it a very desirable choice for analysis, also. However, the higher molecular weight and correspondingly longer retention times of some of these compounds using gas chromatography, such as those for trazodone and risperidone, make the use of HPLC more attractive.

In the case of liquid chromatography, reversed-phase HPLC, using a non-polar column (most often a C8 or C18) and a polar mobile phase, and in which non-polar drugs elute first, is most common, and works well for analysis of basic drugs. For this type of analysis, it is important not only to resolve drugs present in a sample from each other, but also to separate them from the various early-eluting components of a biological matrix. To achieve this, a general rule of thumb is to try to keep retention times within 3-10 minutes for all analytes of interest.

### 1.6. Analytical Methods for Serotonin.

Postmortem stability studies have shown that 5-HT is unstable at room temperature once removed from intact tissue (35-39). Because of this, reagents used for sample preparation must be kept refrigerated, and the samples should be stored at 4 $^{o}$C until

analyzed. Another obstacle associated with the determination of 5-HT in human tissue samples is the fact that all blood has some serotonin, so it is impossible to obtain a true blank for quantitation. If blood bank blood, which is normally used for quantitation of drug found in case samples, is used in this assay, the calibration curve will intercept the y-axis at a point above zero. Because of this, the 5-HT concentration in the "blank" must first be determined in order to accurately quantitate 5-HT in case samples.

Analytical methods for 5-HT in brain tissue (40-42), cerebrospinal fluid (43-45), urine (43), whole blood (45), and plasma (46-48) have all been published. Most of these employ HPLC with either fluorescence or electrochemical detection, because they require little sample handling and provide for fast analysis. GC/MS methods have also been developed for identification of 5-HT in cerebrospinal fluid and brain tissue (49-53). However, the GC/MS methods are generally not sensitive enough for determination of 5-HT and metabolites in platelet-poor or platelet-free plasma. While brain 5-HT concentrations are on the order of several hundred nanomolar, plasma 5-HT levels are usually in the range from 5-15 nanomoles/L.

### 1.7. Research Plan.

The focus of the present research was to develop isolation methods for serotonergic drugs in postmortem specimens in order to accomplish the following goals:

1) Study the role of various serotonergic drugs in fatalities where serotonin syndrome might have played a role using GC/MS and HPLC/PDA methods.

2) Study the postmortem distribution of serotonergic drugs in recent fatalities using the GC/MS and HPLC/PDA methods developed in 1).

3) Determine postmortem serum serotonin levels in fatalities involving obvious overdoses using HPLC with electrochemical detection and compare to levels in subjects where no drug was present in an attempt to elucidate one mechanism of serotonin toxicity.

This research was completed at the Washington State Toxicology Laboratory. All postmortem specimens were obtained from cases submitted to this laboratory for toxicological testing.

## CHAPTER 2. IDENTIFICATION OF TRAMADOL IN BLOOD

### 2.1. Introduction.

Tramadol (Ultram, Ortho-McNeil) (Figure II.1) is a centrally acting, binary analgesic which has been available for use in Europe for several years, although it was only approved for use in the United States in 1995. It was originally introduced in Germany in the late 1970's by Grünenthal as a weak opioid with an atypical

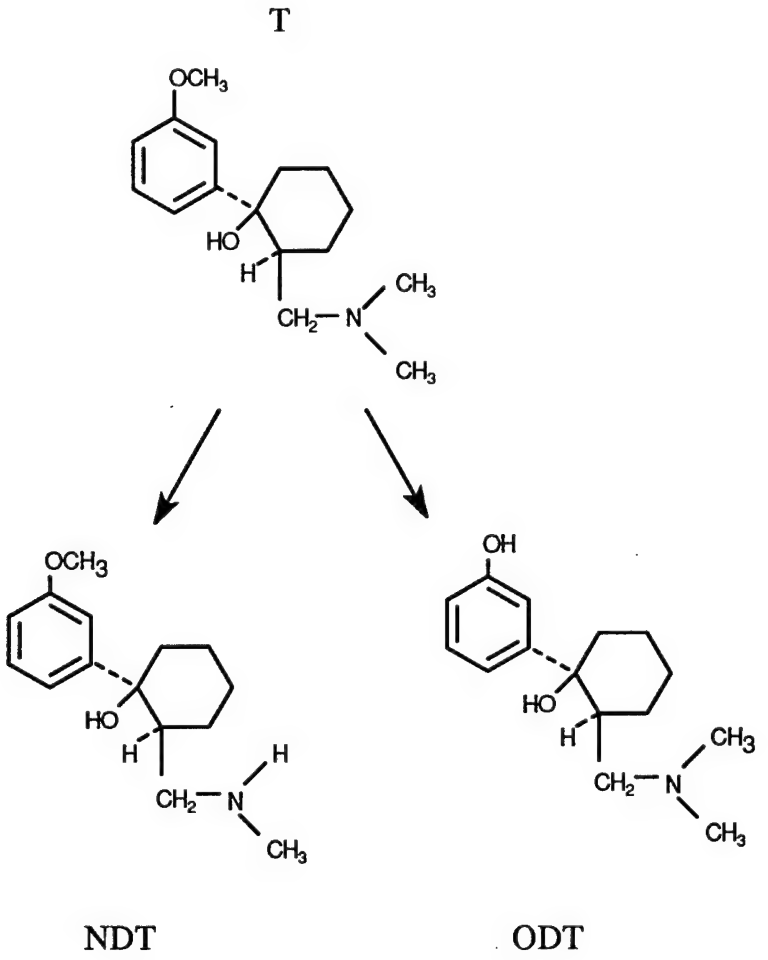



Figure II.1. Structures of tramadol (T), *n*-desmethyltramadol (NDT), and *o*-desmethyltramadol (ODT).

clinical profile (54). The manufacturer claimed that the typical opioid side effects such as respiratory depression or effects on smooth muscles could be lessened or avoided altogether if doses providing analgesic efficacy similar to that of pethidine were used. Because of the (+)-enantiomer's relatively low affinity for the mu-opioid receptor, tramadol was also originally thought to have a low potential for abuse, tolerance, and dependence in treatment up to six months (15). As a matter of fact, despite increasing clinical use, tramadol did not become popular as a drug of abuse up to the early 1990's, and a study published in 1993 found no significant abuse reported with tramadol (55). In studies of physical dependence-producing capacity, tramadol failed to suppress or precipitate withdrawal in morphine dependent mice, rats, and rhesus monkeys (54, 56, 57, 58). However, one study found that tramadol produced mild withdrawal signs when given to morphine dependent, non-withdrawn rhesus monkeys (58), and produced a mild degree of physical dependence following repeated administration to rats, mice, and rhesus monkeys as demonstrated by the production of withdrawal signs following opioid antagonist administration and abrupt drug termination (54, 57, 58, 59). This might be explained by the fact that tramadol's active metabolite, *o*-desmethyltramadol, (ODT) has up to 200 times higher affinity for the mu-opioid receptor and twice the analgesic potency of the parent drug.

Studies suggesting tramadol may have abuse potential have been

performed. Tramadol maintained drug taking in three self-administration studies in lefetamine-trained and drug naive rhesus monkeys (58). In a study of the effects of tramadol in post-addicts to assist in its abuse potential assessment (60), the authors concluded that while the drug does appear to have some potential for abuse, a much larger dose is required to produce subjective effects in patients than that required for morphine.

Ortho-McNeil's recent letter (61) to health care professionals across the country provided new information regarding the potential for abuse, seizures, and anaphylactoid reactions associated with the use of tramadol. The large number of adverse events attributed to tramadol has prompted the company to update the prescribing information for Ultram. Specifically, the new product insert specifies that Ultram is contraindicated in patients with past or present histories of addiction or dependence on opioids, those with allergies to Ultram and/or other opioids, and in those taking other concomitant medications which may reduce the seizure threshold, such as tricyclic antidepressants, other tricyclic compounds, and Selective Serotonin Reuptake Inhibitors (SSRI's).

It is important to consider tramadol's ability to inhibit serotonin reuptake when prescribing the drug for patients already taking drugs with serotonergic activity. It is possible that subjects stabilized on SSRI's or other antidepressants might be susceptible to developing Serotonin Syndrome (SS) upon starting tramadol

therapy. Additionally, it is highly probable that ODT, tramadol's active metabolite, plays a role in fatalities where high concentrations of the metabolite are present, either due to SS, or more likely, to tramadol toxicity. ODT has a higher affinity for the mu-opiod receptor and has twice the analgesic potency of tramadol.

Isoenzyme metabolism is also important to consider in tramadol-related fatalities. CYP2D6, the Cytochrome P-450 isoform for which many SSRI's and tricyclic antidepressants are substrates (1, 23, 62), has been shown to be responsible for metabolism of tramadol to ODT. Consequently, competitive inhibition of 2D6 resulting in enzyme saturation and subsequent lengthened half-life and increased peak plasma concentration may occur when one or more substrates for this isoenzyme are present with tramadol. Such interactions can lead to toxic side effects which may play an important role in tramadol-related fatalities.

Isolation methods for tramadol and its metabolites from blood and urine using gas chromatography/mass spectrometry (GC/MS), gas chromatography with nitrogen-selective detection, and high-performance-liquid chromatography (HPLC) have previously been reported (63-67). However, all of the GC methods involve derivatization of all three compounds prior to analysis. We report analytical methods using GC/MS without derivatization for determination of tramadol and its metabolites. This method was applied to cases of suspected drug-related deaths and drug-

impaired driving.

## 2.2. Materials and Methods.

### 2.2.1. Standards, reagents and solvents.

Tramadol, NDT, and ODT were gifts from R.W. Johnson (Raritan, NJ). The internal standard, papaverine hydrochloride in GC grade methanol, was obtained from Sigma. All other reagents were analytical grade or better and were obtained from Fisher.

### 2.2.2. Gas chromatography.

Gas chromatography/mass spectrometry (GC/MS) was performed on a 5890/5970 GC/MS from Hewlett Packard (Palo Alto, CA). Chromatographic separation was achieved using a 5% phenylmethylsilicone column (30 m x 0.32-micron i.d., Econocap; Alltech). Because of the presence of the phenolic functional group on ODT, concern was raised over whether the polarity of this metabolite would prevent it from eluting from the analytical column. Extracted standards were derivatized with *n*-methyl-bis(trifluoroacetamide) (MBTFA). However, fairly low yields were obtained with derivatization, making it impossible to quantitate samples with concentrations below 1 mg/L for ODT or 0.5 mg/L for NDT. Since the three compounds could be resolved without derivatization, a standard GC/MS method was used. Analyses were performed using a temperature program from 80 to 295$^{o}$C at 15$^{o}$C/min, held at the final temperature for 8 minutes.

### 2.2.3. High performance liquid chromatography.

For high performance liquid chromatographic (HPLC) analysis, a

reversed-phase isocratic method with photodiode array detection (PDA) was used. The system consisted of a Gilson 305/307 pumping unit (Gilson, Middleton, WI), a Rheodyne sample injector (Model 7161, Rheodyne) equipped with a 20-uL loop, a 1040M photodiode array detector (Hewlett Packard), and a Lichrosorb RP Select B column ( 5 um particle size, 250 mm x 4.6 mm I.D., Merck; Alltech). A flow rate of 1.5 ml/min was used, and data were collected in peak capture mode from 190 to 400 nm, with pilot wavelengths at 260 nm/230 nm.

In order to optimize HPLC analytical method parameters as efficiently as possible, the experimental design outlined in Table II.1 was used. Five different analytical columns (C8, C18, CN, Cyclobond III alpha, and Merck RP Select B) were used to separate tramadol from both major metabolites using the mobile phase combinations of acetonitrile (AcN) and 0.05M phosphate buffer (pH 3) in the amounts listed.

Resolution is expressed as a value between zero and one, and is calculated using the following formula (68):

$$R_s = 2(t_{R,2} - t_{R,1})/(W_1 + W_2)$$

Where $R_s$ = resolution (unitless)

$t_{R,1}$ = the retention time for analyte 1 (minutes)

$t_{R,2}$ = the retention time for analyte 2 (minutes)

$W_1$ = peak width of analyte 1 (minutes)

$W_2$ = peak width of analyte 2 (minutes)

In these analyses, analyte 1 was NDT and analyte 2 was ODT.

Tramadol was resolved from both metabolites on each column. However, the only column/mobile phase combinations which

Table II.1. Experimental Design for HPLC Method Optimization.

| Run | C8 | C18 | CN | CycIII Alpha | RP Sel B |
|---|---|---|---|---|---|
| 1 | 5% AcN | --- | --- | --- | --- |
| 2 | 10% AcN | --- | --- | --- | --- |
| 3 | 15% AcN | --- | --- | --- | --- |
| 4 | 20% AcN | --- | --- | --- | --- |
| 5 | 40% AcN | --- | --- | --- | --- |
| 6 | --- | 5% AcN | --- | --- | --- |
| 7 | --- | 10% AcN | --- | --- | --- |
| 8 | --- | 15% AcN | --- | --- | --- |
| 9 | --- | 20% AcN | --- | --- | --- |
| 10 | --- | 40% AcN | --- | --- | --- |
| 11 | --- | --- | 5% AcN | --- | --- |
| 12 | --- | --- | 10% AcN | --- | --- |
| 13 | --- | --- | 15% AcN | --- | --- |
| 14 | --- | --- | 20% AcN | --- | --- |
| 15 | --- | --- | 40% AcN | --- | --- |
| 16 | --- | --- | --- | 5% AcN | --- |
| 17 | --- | --- | --- | 10% AcN | --- |
| 18 | --- | --- | --- | 15% AcN | --- |
| 19 | --- | --- | --- | 20% AcN | --- |
| 20 | --- | --- | --- | 40% AcN | --- |
| 21 | --- | --- | --- | --- | 5% AcN |
| 22 | --- | --- | --- | --- | 10% AcN |
| 23 | --- | --- | --- | --- | 15% AcN |
| 24 | --- | --- | --- | --- | 20% AcN |
| 25 | --- | --- | --- | --- | 40% AcN |

resolved NDT and ODT to any extent were the CN column using 5 and 10% acetonitrile, the Cyclobond III alpha column using 5% acetonitrile, and the RP Select B column using 10 and 15%

acetonitrile. As shown in Figure II.2, the RP Select B column using 10% acetonitrile by far gave the best resolution of the two metabolites ($R_s$ = 0.87).

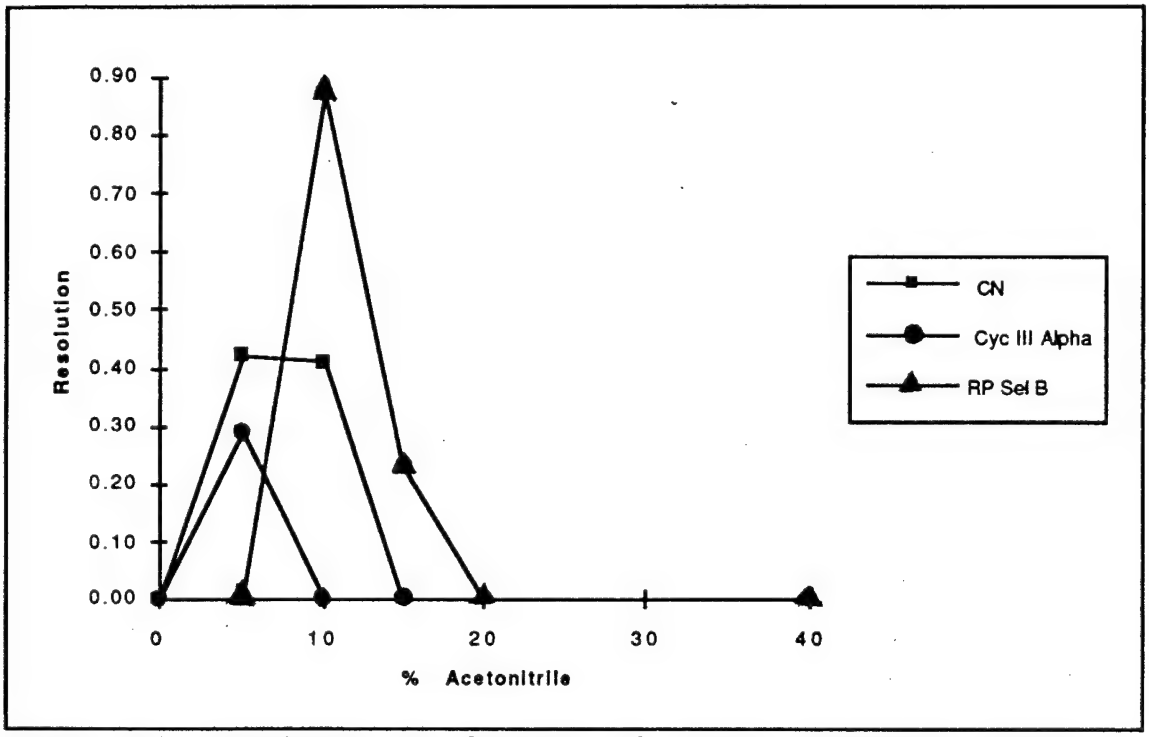



Figure II.2. Resolution as a function of column and mobile phase.

### 2.2.4. Case sample analysis.

Blood samples collected at autopsy during the investigation of twelve unrelated fatalities were each placed in separate 10-ml vials containing sodium fluoride and potassium oxalate (Vacutainer; Becton Dickinson, New Jersey). The samples were refrigerated until analysis was performed. Since derivatization was not required, liquid-liquid extractions were performed using a procedure based on that described by Foerster and co-workers (69,

70) which has been modified for general use in the Washington State Toxicology Laboratory for screening basic drugs. Blood (1 ml), internal standards (diphenylamine and papaverine, 100 ul of 1-mg/L and .5 mg/L solutions, respectively), and pH 9 saturated potassium borate buffer (1 ml) were mixed and extracted with *n*-butyl chloride (3 ml) after centrifuging at 2000 rpm for 5 minutes. The organic fraction was back extracted into 3M hydrochloric acid (200 ul), which was then basified with concentrated ammonium hydroxide and reextracted into chloroform (100 ul). The resulting solution was then analyzed by GC/MS.

**2.3. Results.**

Figure II.3 shows liquid chromatographic separation of tramadol from both metabolites. As shown in Figure II.4, tramadol, NDT, and ODT were resolved from both internal standards, papaverine and diphenylamine in GC/MS analysis. Mass spectra of the three analytes are shown in Figure II.5. Periodically, variable amounts of an artifact of NDT appeared both in samples from patients and quantitative standards with molecular ion m/z 261 (Figure II.6) (the molecular weight of NDT is 249). This was investigated by the drug's manufacturer (71) who conducted proton- and $^{13}C$- nuclear magnetic resonance spectroscopy of the standard and concluded that the m/z 261 peak corresponds to a carbamate derivative of NDT, presumably formed in the injection port of the GC. The variable nature of this phenomenon might affect reliability of quantitative results from NDT. However, this should not affect

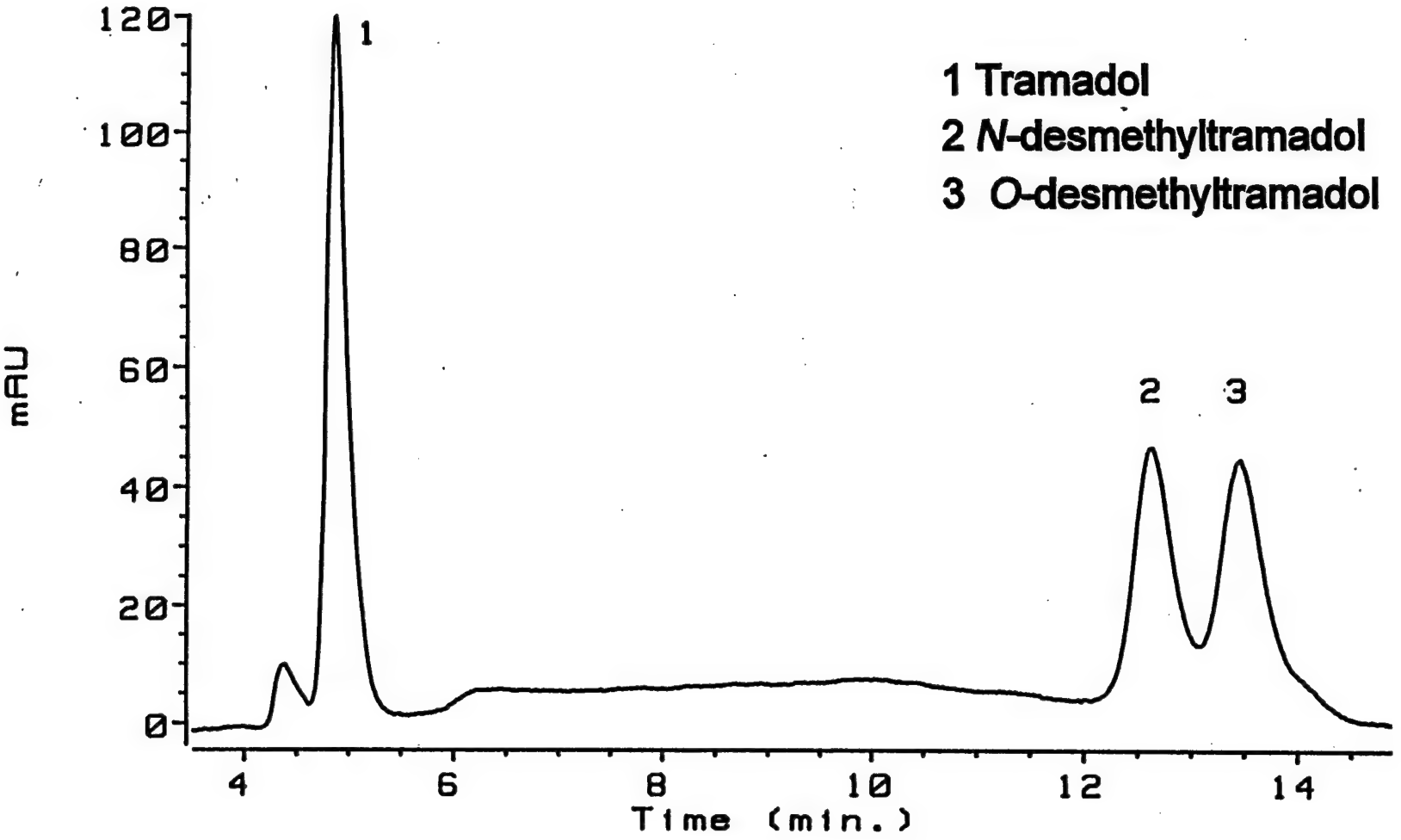



**Figure II.3. Liquid chromatography - separation of tramadol and metabolites.**

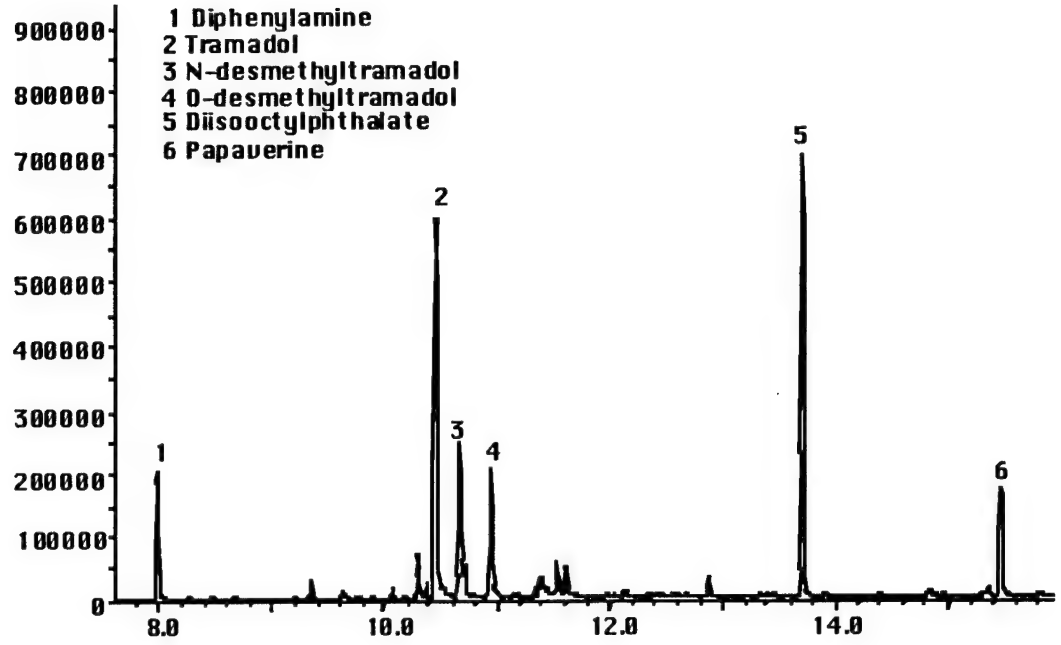



Figure II.4. Gas chromatographic separation of tramadol, NDT, and ODT from internal standards.

interpretation since NDT is an inactive metabolite.

The HPLC method was not used for quantitation. Even though good separation of the two metabolites was obtained, the long waiting time between elution of tramadol and of the two major metabolites made it a less desirable method.

The GC/MS method was linear for tramadol and both metabolites over the range 0.01- 10 mg/L, with regression coefficients for tramadol, NDT, and ODT of 0.996, 0.993, and 0.990, respectively. Limits of detection (LOD) and quantitation (LOQ) were 0.01 and 0.02 mg/L, respectively, and were determined in the following manner. Replicate analyses of tramadol and metabolite standards at concentrations ranging from 0.01-10.00 mg/L were performed,

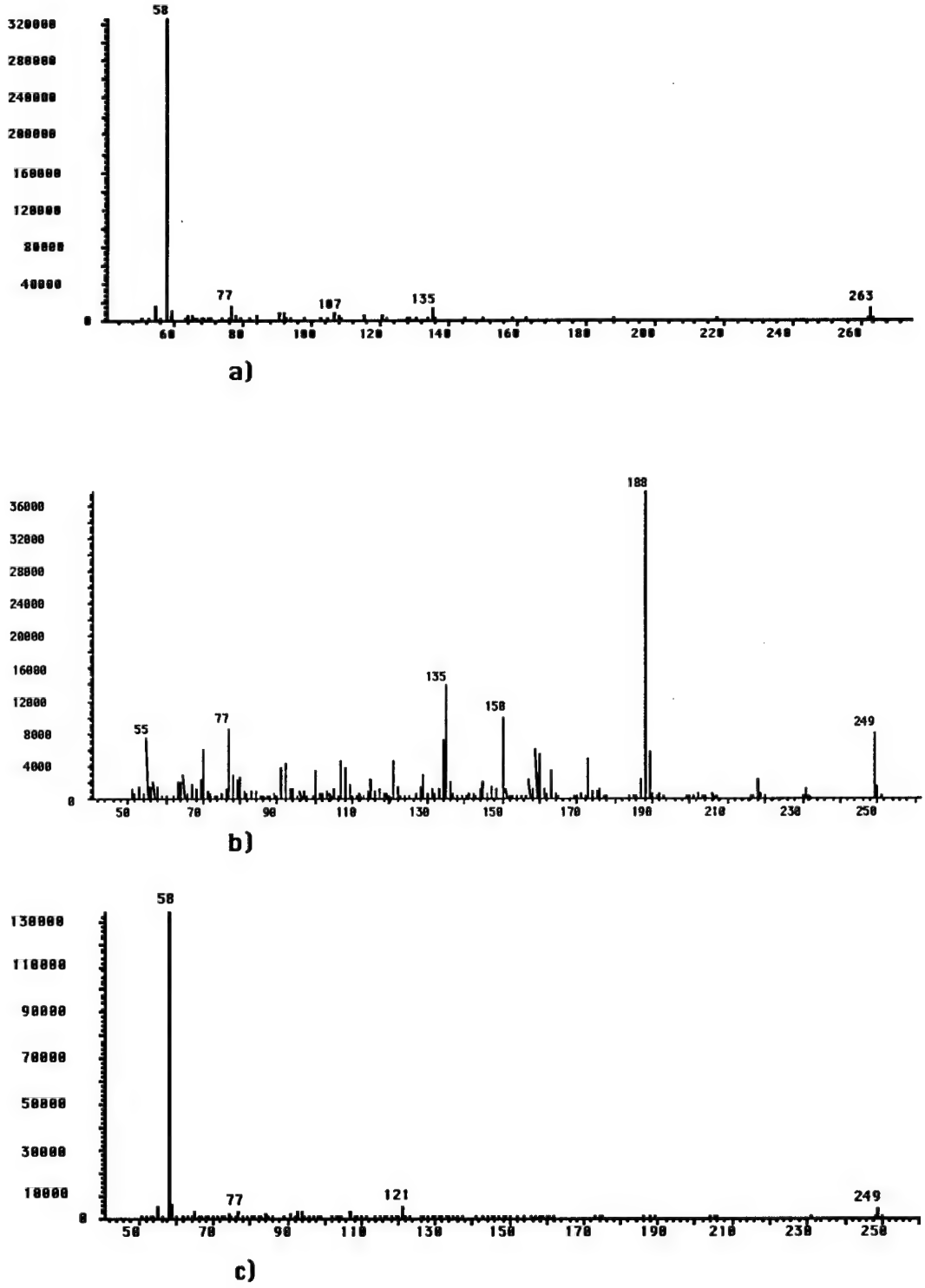



Figure II.5. Mass spectra for tramadol (a), *n*-desmethyltramadol (b), and *o*-desmethyltramadol (c).

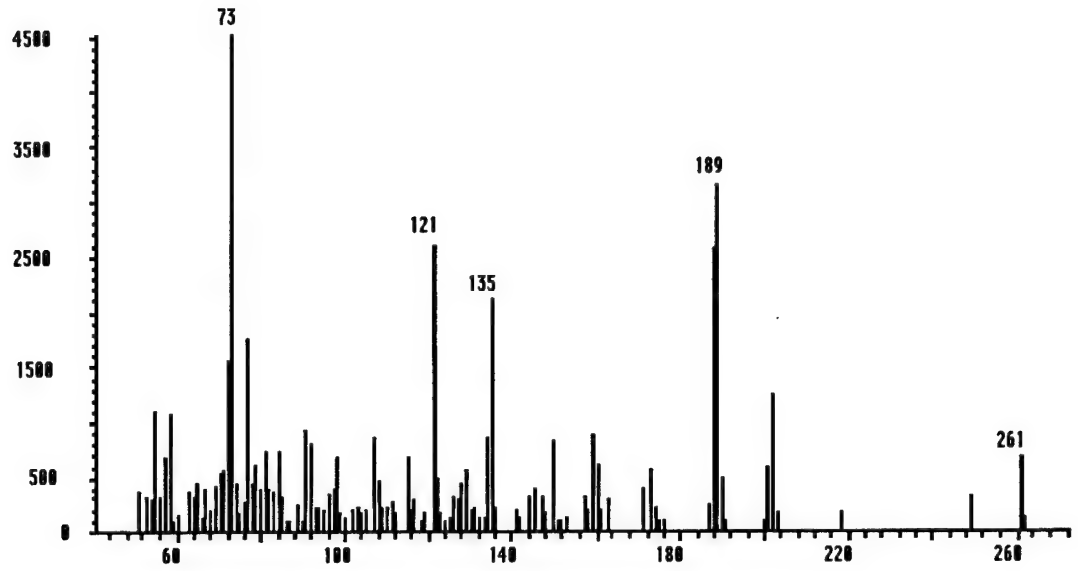



Figure II.6. Mass spectrum of *n*-desmethyltramadol artifact.

and the average concentration and standard deviation determined from these results. Standard deviation was then plotted as a function of concentration (see Figure II.7), and the y-intercept taken to be $SD_o$. To determine the LOD and LOQ for each compound, the equations

$$LOD = 3 \times SD_o$$

$$LOQ = 8 \times SD_o$$

were used. Concentrations of tramadol, metabolites, and other drugs found in each case, as well as cause and manner of death are shown in Table II.2.

## 2.4. Discussion.

In clinical trials, peak plasma levels for tramadol and ODT, which were reached within two and three hours of administration of a

single 100-mg dose, were 0.306 ± 0.078 and 0.055 ± 0.020 ug/ml, respectively. Within two days of 100-mg Q.I.D. dosing, steady

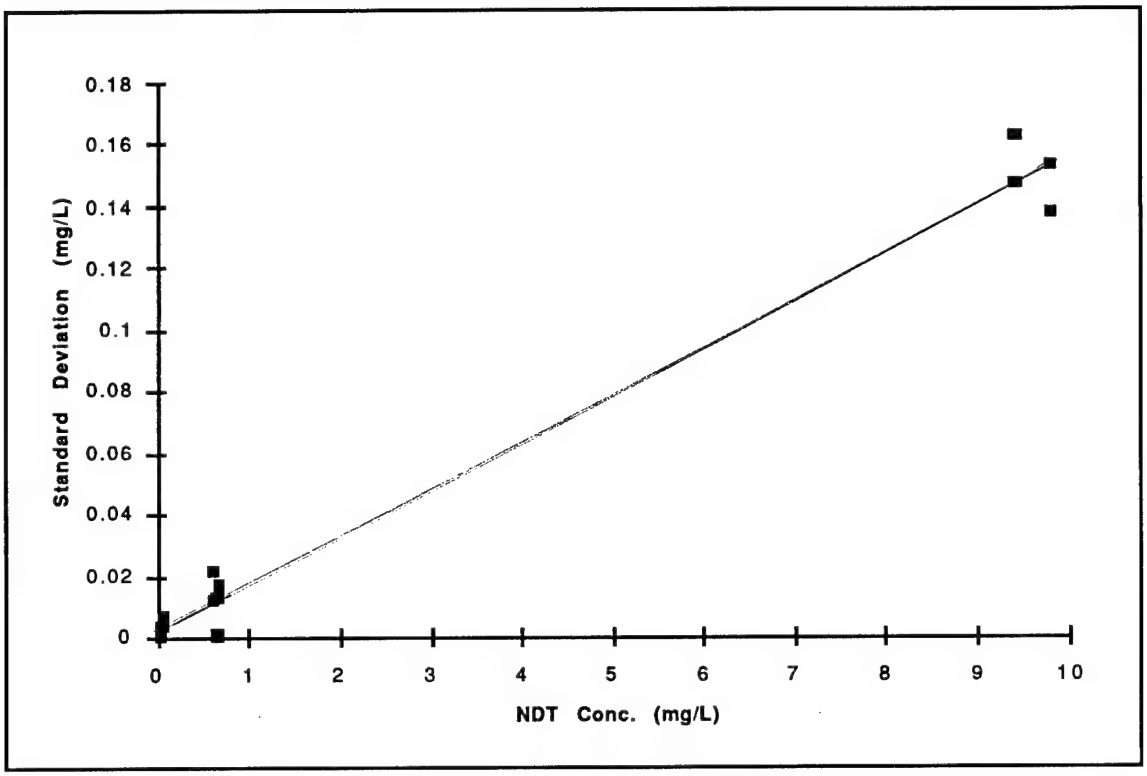



Figure II.7. Standard deviation as a function of NDT concentration.

state tramadol and ODT plasma levels (0.592± 0.177 ug/ml) have been reported (72). Tramadol and ODT have half-lives of 6.3 and 7.4 hours, respectively, and tramadol is only 20% plasma bound. Sixty percent of the dose is excreted in urine as metabolites, with the rest eliminated as unchanged drug. There has been only one tramadol- related fatality reported in the literature, although the

## Table II.2. Case Information on Subjects Testing Positive for Tramadol.

| Case | Age | Sex | T (mg/L) | ODT (mg/L) | NDT (mg/L) | Alcohol (g/100 mL) | Other Drug Use (mg/L) | Circumstances Surrounding Death | Cause of Death | Manner of Death |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 18 | F | 0.03 | 0.06 | 0.11 | neg | carbon monoxide (<5% sat) nicotine/cotinine | subject was poorly nourished w/ below normal exercise, history of alcohol & drug abuse; became short of breath & collapsed after mild exertion, unable to be resuscitated | sudden cardiac death from unknown etiology | undetermined |
| 2 | 41 | M | 0.08 | 0.07 | 0.03 | 0.02 | morphine (0.18) | history of drug abuse, found dead by friend during camping trip; had ingested large quantities of alcohol, heroin, & pain medication | acute intravenous narcotism | accident |
| 3 | 48 | M | 0.41 | 0.04 | 0.27 | neg | morphine (0.14) propoxyphene (0.04) norpropoxyphene (0.23) | history of drug abuse, died suddenly & unexpectedly | acute substance abuse | undetermined |
| 4 | 54 | M | 0.05 | 0.04 | 0.01 | 0.06 | morphine (0.275) codeine (0.07) norpropoxyphene (<0.05) nordiazepam (0.16) | history of emphysema & drug abuse; found dead w/ drug paraphernalia at scene; autopsy findings incl. pulmonary edema, arteriosclerosis, & atherosclerosis | acute morphine intoxication | undetermined |
| 5 | 28 | F | 0.05 | 0.46 | 0.73 | neg | dextromethorphan (0.90) propoxyphene (0.40) norpropoxyphene (0.90) morphine (<0.025) | history of drug abuse; died suddenly & unexpectedly after ingesting morphine & various pain medications; drug paraphernalia found at scene; autopsy revealed pulmonary edema | multiple drug abuse | undetermined |

Table II.2 (Continued).

| Case | Age | Sex | T (mg/L) | ODT (mg/L) | NDT (mg/L) | Alcohol (g/100 mL) | Other Drug Use (mg/L) | Circumstances Surrounding Death | Cause of Death | Manner of Death |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 57 | F | 0.48 | 0.02 | 0.32 | neg | doxepin (10.00) desmethyldoxepin (1.4) nicotine/cotinine | history of depression, on medication for same, found deceased at home; empty pill bottles (Doxepin, Paxil, Lodine) found in bedroom | acute doxepin overdose | undetermined |
| 7 | 36 | M | 0.16 | 1.84 | 0.05 | neg | alprazolam (0.11) propoxyphene (1.9) norpropoxyphene (3.0) | history of back pain, found dead in parked motor vehicle w/ upper torso slumped towards passenger seat; Ambien (zolpidem tartrate) found on right passenger seat | acute combination drug intoxication | probable accident |
| 8 | 58 | F | 0.12 | 0.28 | 0.60 | 0.10 | amitriptyline (0.13) nortriptyline (<0.1) | subject died at home after 2 days discharged from hospital, on Inapsine IV given by daughter, but investigators unable to find same; coroner delayed decision to do autopsy and body not refrigerated so moderately decomposed | coronary artery disease, although determination made prior to receipt of toxicology results | undetermined |
| 9 | 39 | M | 1.43 | 0.03 | 0.18 | neg | amitriptyline (0.59) nortriptyline (0.68) diazepam (0.27) nordiazepam (0.19) | no known medical history, found dead, face down, at home; prescription drugs incl. prednisone, diazepam, tramadol, and methocarbamol | positional asphyxia secondary to acute drug intoxication | accident |

Table II.2 (Continued).

| Case | Age | Sex | T (mg/L) | ODT (mg/L) | NDT (mg/L) | Alcohol (g/100 mL) | Other Drug Use (mg/L) | Circumstances Surrounding Death | Cause of Death | Manner of Death |
|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 38 | F | 2.47 | 0.06 | 0.16 | neg | amitriptyline (0.43) nortriptyline (0.71) hydrocodone (0.07) | died suddenly & unexpectedly; Rx include Ultram, Amitriptyline, Deltazone, Buspar | acute tramadol intoxication | undetermined |
| 11 | 32 | M | 22.59 | 0.34 | 2.08 | 0.02 | propranolol (3.90) desipramine (1.00) trazadone (3.70) | admitted to ER due to "multiple drug overdose-tricyclic & betablocker", died shortly after admission; HIV+; Rx include Propranolol, Ultram, Trazadone, Klonopin, Desipramine, Hydroxyz, Lithonate | multiple drug overdose | suicide |
| 12 | 44 | M | 1.13 | 0.46 | 0.3 | 0.14 | nordiazepam (0.20) carisoprodol (2.70) meprobamate (5.90) | released from mental hospital in morning & walked to train tracks after leaving friend's house; placed head on tracks when he heard train coming; engineer was unable to stop in time | decapitation | suicide |

extent to which tramadol contributed to the death is unclear, as high concentrations of other drugs were present (24). In that case, the authors suspected the involvement of serotonin syndrome as a result of moclobemide-clomipramine interaction as has been previously reported (73). The authors further suggested that tramadol could have had a synergistic effect on the serotonin syndrome, due to its serotonin reuptake inhibiting ability. Other fatalities involving tramadol have occurred (74) although the cases have not been published. The tramadol levels in these cases were 2.7 mg/L and 1.3 mg/L; however, metabolite concentrations were not measured, and it is unknown whether other drugs were involved.

The presence of other drugs seems to be typical of cases involving tramadol. For example, three drug-impaired drivers, all of whom were found to have therapeutic levels of tramadol in their blood, and one who had toxic tramadol levels in her urine, also had other drugs present. Table II.3 summarizes these cases.

As was mentioned earlier, tramadol is metabolized to ODT by CYP2D6, and as such is subject to metabolic inhibition, which may affect plasma levels of the parent drug. The presence of other substrates for this isozyme should therefore be taken into consideration in cases involving tramadol. The therapeutic effect of the drug might be increased or decreased by the presence of a 2D6 inhibitor, as both tramadol and ODT are pharmacologically active.

Table II.3. Tramadol Levels in Four Drug-impaired Drivers.

| Age | Sex | T (mg/L) | ODT (mg/L) | NDT (mg/L) | Other Drugs Present (mg/L) | Case History |
|---|---|---|---|---|---|---|
| **Blood** | | | | | | |
| 44 | M | 0.07 | 0.05 | 0.09 | cocaine (<0.1) diazepam (<0.1) nordiazepam (<0.1) propoxyphene (<0.1) norpropoxyphene (0.31) benzoylecgonine (pos) | Hx of open heart surgery, marijuana use; admitted taking marijuana, darvocet, voltaren |
| 39 | F | 0.17 | 0.11 | 0.04 | morphine (<0.01) | Hx of heroin abuse; admitted taking heroin, diazepam, & dextromethorphan |
| 39 | F | 0.29 | 0.05 | 0.03 | methadone (0.09) | Stopped on return from methadone clinic; told police she had just been given methadone |
| **Urine** | | | | | | |
| 40 | F | 31.37 | 4.32 | 6.51 | ethanol (0.1 g/100 ml) methamphetamine amphetamine | Stopped for erratic driving; prescription bottle of Ultram found in subject's car |

Consequently, it is important to consider ODT levels when interpreting tramadol levels. Comparing the levels of the parent drug and metabolite might provide information as to whether the drug was chronically or acutely ingested. Also, ODT contributes to toxicity by way of its analgesic activity, which is twice as high as the parent compound. Its higher mu-opioid receptor affinity also contributes to toxicity through depression of the central nervous system.

The role of tramadol in fatalities is difficult to determine because there have been no fatal overdoses reported where tramadol alone was present. However, an investigation of the circumstances surrounding each case in this study will provide investigators with baseline information regarding the blood levels associated with different types of fatalities. No ante-mortem medical evaluation was performed on any of the subjects described here, so consideration of the role of Serotonin Syndrome in these fatalities was based solely on the presence or lack of serotonergic activity of all drugs present. The therapeutic and toxic ranges for drugs present in the blood samples are summarized in Table II.4, in addition to their relative ability to inhibit neurotransmitter reuptake and the CYP450 isozyme(s) (if known) for which they are substrates (1, 23, 62, 75, 76).

The low levels of tramadol and metabolites in Case 1 make it unlikely that the drug played a role in this death. Similarly, the very low carbon monoxide levels rule out smoke inhalation as a

cause of death. The true cause of the subject's sudden death remains undetermined.

Table II.4. Properties of Drugs Found in Postmortem Blood

| Drug | Therapeutic conc, mg/L* | Toxic conc, mg/L* | Reuptake Inhibition NE | DA | ST |
|---|---|---|---|---|---|
| alprazolam (3A4, 2D6 minor) | 0.02-0.04 | >0.07 | 0 | 0 | 0 |
| amitriptyline (2D6,3A4 minor) | 0.06-0.22 | >1.0 | ± | 0 | ++ |
| codeine (2D6 - to morphine) | 0.03-0.34 | 1.00-8.80 | 0 | 0 | 0 |
| desipramine (3A4; inhibits 2D6) | 0.01-0.28 | 1.20-15.00 | +++ | 0 | 0 |
| dextromethorphan (2D6, 3A4 minor) | 0.38 | LD = 0.5 g | 0 | 0 | + |
| doxepin | 0.03-0.15 | >0.1 | ± | 0 | + |
| diazepam (2C19) | 0.1-2.5 | >1.5 | 0 | 0 | 0 |
| hydrocodone (2D6) | 0.002-0.024 | 0.13-7.00 | 0 | 0 | 0 |
| morphine | 0.01-0.07 | 0.12-4.70 | 0 | 0 | 0 |
| propoxyphene (2D6) | 0.05-0.75 | 1.0-2.0 | + | 0 | + |
| propranolol (2D6) | 0.01-0.26 | 4.00-29.00 | 0 | 0 | 0 |
| tramadol (2D6) | 0.23-0.77 | LD = 0.5 g | + | 0 | + |
| trazodone | 0.49-1.60 | >15.00 | 0 | 0 | + |

Abbreviations and symbols: NE = norepinephrine; DA = dopamine; ST = serotonin; + to ++ = active to strongly active; ± = weakly active; 0 = lacking

* All levels come from Baselt (22) except those for tramadol, which come from the revised product insert from Ortho-McNeil (76)

Cases 2, 3, and 4 are clearly attributable to acute morphine intoxication (22). In each case, the morphine concentration is quite high, whereas the levels of other drugs present, including tramadol, are well within or below the therapeutic window.

However, it is possible that tramadol may have contributed slightly to these deaths, due to its mu-opioid receptor affinity. The occurrence of sudden death, as in Case 3, and the autopsy finding of pulmonary edema, as in Case 4, are both common in morphine overdoses (77). It is unclear how these subjects obtained tramadol, although the drug is not a scheduled narcotic. Tramadol's abuse potential was originally reported to be much lower than that of morphine (72). However, Ortho-McNeil's recent letter to health care professionals providing additional information on this subject reports one-hundred-fifteen spontaneous domestic adverse events described as drug abuse, dependence, withdrawal, or intentional overdose, not including cases of accidental overdose. Patients with a past or present history of addiction or dependence on opioids account for a majority of these reports (61). The fact that four of the twelve cases reported here involving tramadol were also positive for morphine suggests that the manufacturer's concerns are well founded.

Morphine levels reported as less than 0.025 ug/ml as in Case 5 mean that the sample tested positive, although the level is below the limit of quantitation of the analytical method. Blood morphine levels in morphine-caused deaths can be significantly lower when the survival period is longer than 3 hours (77).

The tramadol level in Case 5 was low, but its effect combined with that of ODT may have contributed to the opioid effect of morphine. Further, the presence of dextromethorphan and

propoxyphene, both CYP2D6 substrates, might have contributed to decreased clearance of tramadol due to competitive metabolic inhibition, thereby prolonging or enhancing tramadol's effects. In addition, it is conceivable that the serotonin reuptake inhibiting ability of both dextromethorphan and propoxyphene, combined with that of tramadol played an important role in this subject's death.

Case 6 is clearly attributable to doxepin overdose. However, interaction from tramadol could contribute to cause of death due to the moderate serotonergic effect of both drugs. The levels of tramadol and metabolites are within therapeutic ranges, but concomitant administration with very high levels of doxepin might have contributed to serotonergic crisis, due to the chronotropic and inotropic effects of serotonin, as well as the increases vasoconstriction produced by norepinephrine, angiotensin II, and histamine (3).

Case 7 is interesting, in that although the tramadol concentration is fairly low, the ODT concentration is extremely high, and propoxyphene and alprazolam, both CYP2D6 substrates, were also present at fairly high concentrations. While it is possible that a metabolic interaction took place, the likely explanation is that the subject ingested a large but not fatal dose of tramadol at an earlier time. Acute combined drug intoxication seems reasonable as a cause of death.

Case 8 was certified as a natural death before toxicological

testing was complete. In reviewing the toxicology results, however, it is possible that there would be some serotonergic interaction between the drugs present, and that, together with the alcohol present might have played a role in the death.

The very high tramadol levels and low metabolite concentrations in Case 9 point to an acute tramadol ingestion in a combined drug overdose. Again, the effect of elevated concentrations of several serotonergic drugs which are also 2D6 substrates might have contributed to increased plasma concentrations which played a role in this fatality.

The presence of amitriptyline and hydrocodone, both substrates for CYP2D6, indicates a possible competitive inhibition of the isoenzyme. The high concentration of tramadol relative to ODT further supports this. Also, it is conceivable that the combined serotonergic activity of amitriptyline and tramadol was significant in this person's death.

In addition to significant concentrations of several substrates of 2D6 in Case 11, high levels of desipramine, an inhibitor of 2D6, were also present. As in Case 10, extremely high levels of tramadol relative to those of its metabolites support the possibility of competitive inhibition. It is likely that the serotonergic activity of several drugs present in the subject's blood also played an important role.

The concentrations of tramadol and ODT in Case 12 are both well above the therapeutic range, and it, along with the high blood

alcohol level, might have contributed to central nervous system depression. However, decapitation was clearly the cause of death in this case.

In summary, tramadol is usually found to be present with antidepressants and/or other opiates, both of which can interact with tramadol and its active metabolite when the use of the drug is indicated. ODT levels should be taken into consideration when interpreting tramadol concentrations, especially when morphine is also present. The appearance of the two drug interactions discussed here, competitive inhibition of CYP2D6 leading to decreased clearance of tramadol and potentially toxic side effects, and combined serotonergic activity of two or more drugs, should also be considered when interpreting tramadol levels. In the subjects in these cases who had histories of heart disease, it is possible that increased serotonin levels might have contributed to their deaths due to a combination of increased serum serotonin levels and the decreased ability of the endothelium to metabolize serotonin due to ischemic damage as discussed in Chapter 1 (25).

## 2.5. Conclusion.

Tramadol is a synthetic, centrally acting, binary analgesic used as a racemic mixture which is not chemically related to nonsteroidal anti-inflammatory nor opiate-derived drugs. Its enantiomers have affinity for mu-opioid, serotonin, and norepinephrine receptors. Tramadol is metabolized by CYP2D6 to ODT, the only active metabolite, which has both higher affinity for the mu-opioid

receptor and more potent analgesic effect than the parent drug. Tramadol's other primary metabolite is NDT. Approximately 30% of the dose is excreted as unchanged drug, 60% as the two major metabolites, and the remainder as unidentified or unextractable metabolites.

Tramadol is usually present in fatalities in combination with antidepressants and/or other opiates. This is important because of the two types of drug interactions described in this chapter: saturation of 2D6 resulting in increased plasma tramadol concentrations, and combined serotonergic effect. Most antidepressants have some serotonergic activity and are substrates for 2D6, as are some opiates. The presence of tramadol with other opiates indicates its abuse potential may in fact be much higher than originally thought. For this reason, clinicians and physicians alike should watch for evidence of tramadol abuse, and should attempt to determine if patients are taking other agents with mu-opioid receptor affinity.

Toxicity due to tramadol use is dependent on other drugs present. In the cases analyzed in this study involving morphine, blood levels of tramadol tended to be inversely proportional to morphine levels, such that in clear cases of morphine toxicity the tramadol levels were sometimes below the therapeutic window, and may be inconsequential. In such cases, however, careful consideration of the ODT level must be given. This metabolite's higher affinity for the mu-opioid receptor and increased analgesic

potency might contribute to morphine toxicity when its levels are substantially above the therapeutic range (i.e.≥0.10 mg/L).

In cases where antidepressants or antianxiety agents are also present, tramadol and ODT concentrations are likely to be within or slightly above the therapeutic range (0.23-1.20 mg/L for tramadol, 0.04-0.10 mg/L for ODT). For example, the subject who was decapitated in Case 12 had a tramadol level of 1.13 mg/L and ODT level of 0.46 mg/L in his blood, but these high concentrations did not kill him. In contrast, cases of clear tramadol toxicity tend to have tramadol concentrations far above therapeutic levels (>1.20 mg/L), with ODT concentrations at or below the therapeutic range (< 0.08 mg/L). However, even in clear tramadol overdoses, other drugs are still likely to be present, and their albeit minor contribution to fatality should not be ignored.

# CHAPTER 3: ATYPICAL ANTIDEPRESSANTS AND METABOLITES IN POSTMORTEM BLOOD

## 3.1. Introduction.

The recognition of multiple 5-HT receptors in various areas of the body has engendered a variety of new, more specifically acting drugs for use as antidepressants in the effort to find effective medications with minimal side effects, such as selective serotonin reuptake inhibitors (SSRI's), or other drugs which do not fit easily into the category of SSRI or tricyclic antidepressant (TCA), such as trazodone and bupropion. The standard TCA's are generally associated with side effects such as sedation, anticholinergic effects (dry mouth, constipation, blurred vision, and urinary retention), and cardiovascular effects (hypotension, intracardiac conduction, and slowing). This range of side effects stems from the number of different receptors targeted by TCA's, and from the fact that the tertiary amine TCA's are demethylated *in vivo* to secondary amines, which are potent blockers of norepinephrine reuptake (13). In addition to the larger side effect profile, there is a significant lack of response in a large percentage of patients on TCA's, the therapeutic ranges are fairly narrow, and they have limited utility as research tools for understanding pathophysiology of clinical depression due to their multiple sites of action.

Fluoxetine, sertraline, paroxetine, and venlafaxine are all becoming more frequently prescribed to treat a variety of affective disorders. Fluvoxamine was only approved for use in the United

States in 1995 (78), and has been prescribed primarily to treat obsessive-compulsive disorder. Due to its relatively recent arrival on the market, fatalities involving fluvoxamine have not been reported except by the manufacturer, Solvay.

Trazodone and its newer congener, nefazodone, are atypical antidepressants with some serotonergic activity, although less than the SSRI's. Since its release, trazodone has been widely prescribed, occupying approximately one-third of the market for antidepressants in the United States. Its combination of sedation without anticholinergic effects has made trazodone especially attractive in older patients who may be medically compromised or otherwise sensitive to the anticholinergic actions of the standard TCA's. Preclinically, trazodone was shown to have mixed agonist/antagonist activity on serotonergic systems. However, it has been shown to be a potent serotonin agonist *in vivo*, which is possibly due to the appearance of substantial concentrations of trazodone's major metabolite, m-chlorophenylpiperazine (mCPP), a potent serotonergic agonist, at higher doses of the parent drug (13). MCPP has a longer half-life than trazodone, and has been studied for use as a pharmacological probe of the serotonin system.

The monoaminergic antagonism caused by occupation of central 5-$HT_2$ receptors by SSRI's, trazodone and nefazodone, causing immediate blockade of serotonin reuptake, is believed to be responsible for the antidepressant effect of these drugs, which can take several weeks to appear. The brain reacts to the first dose by

reducing serotonergic activity for more than 24 hours (79), which results in decreased concentration and accumulation of 5HIAA, decreased accumulation of 5-hydroxytryptophan after decarboxylase inhibition, decreased disappearance of 5-HT after tryptophan hydroxylase inhibition, decreased incorporation of new L-tryptophan into 5-HT and 5HIAA, and decreased firing of the 5-HT neurons in the raphe nuclei. These effects result in an increase of extracellular serotonin of up to 286% (79) due to increased 5-HT concentrations in the synaptic cleft and increased activation of the postsynaptic receptors.

SSRI's have become more popular than the TCA's in the treatment of a variety of affective disorders due to their specificity of action. This specificity allows physicians to titrate a drug against a particular disorder, simultaneously increasing the possibility of effective treatment, decreasing the possibility of untoward side effects, and increasing general knowledge regarding conditions which may be treated successfully with the drug.

However, a new constellation of potentially toxic side effects have been linked to SSRI use. Specifically, brainstem and spinal cord activation of the 5-$HT_{1A}$ receptor resulting from SSRI occupation of the central 5-$HT_2$ receptors has been implicated in the literature in the development of Serotonin Syndrome (SS), as discussed in Chapter 1 (11). Interaction with dopamine and 5-$HT_2$ receptors may also be involved, and it has recently been hypothesized that SS may be mediated by presynaptic inhibition of

dopamine release or synthesis, in a manner similar to neuroleptic malignant syndrome (NMS) (80). Diagnosis of SS as opposed to NMS is largely one of exclusion and can be made by noting the non-presence of neuroleptic drugs, such as chlorpromazine or reserpine. The accepted treatment for NMS is to give monoamine oxidase inhibitors (MAOI's), which increase serotonin levels by inhibiting its metabolism. When the non-presence of neuroleptic drugs is overlooked, therefore, symptoms of SS can be worsened. The appearance of hyperthermia [temperature >40.5°C (105°C)] in patients who have developed other symptoms of SS is indicative of a severe, potentially fatal disease process (80). Deaths due to either NMS or SS may also occur due to the presence of predisposing factors, such as peripheral vascular disease, environmental hyperthermia, or seizure disorder (11, 25, 80).

Another potentially fatal side effect associated with many of the second generation atypical antidepressants occurs in subjects with heart disease. Increased serum serotonin levels due to the presence of drugs which inhibit 5-HT reuptake may play an important role in fatalities involving these drugs due to the contracting action of platelet 5-HT, as explained in Chapter 1 (3-5).

As with tramadol, isoenzyme metabolism can play an important role in fatalities involving these drugs and should be considered when interpreting blood levels. Because many serotonergic drugs are substrates for either CYP2D6 or 3A4, or both, and are often found in combination, enzyme saturation due to competitive

inhibition can lead to increased blood concentrations which can in turn lead to toxic side effects.

Bupropion is a potent inhibitor of dopamine reuptake, and it also weakly blocks norepinephrine. It may act to potentiate the serotonergic effects of SSRI's due to interaction with dopamine receptors. Patients taking bupropion in combination with SSRI's may therefore be at risk of developing serotonin syndrome, although there is likely some other as yet unidentified factor causing some patients to develop worse cases of the syndrome than others.

A number of drug overdose fatalities involving either sertraline, fluoxetine, venlafaxine, trazodone, or bupropion have been reported in the literature over the past few years (11, 81-99, 118). In this chapter, analytical methods using gas chromatography/mass spectrometry and high performance liquid chromatography with photodiode array detection suitable for determination of a range of common atypical antidepressants and their active metabolites are discussed. These methods were applied to cases of suspected drug-overdose deaths, and the results of these analyses and the probable role of these drugs in the fatalities is investigated. Table III.1 details the structure, relative receptor affinity, and metabolites of the drugs discussed in this paper. In all, fifty-two postmortem cases were analyzed.

Table III.1. Structure, Half-life ($t_{1/2}$), Volume of Distribution ($V_d$), and Metabolites of Drugs Studied.

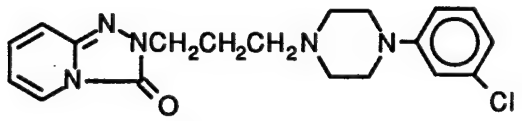
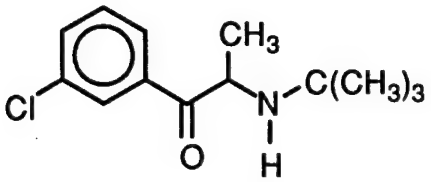
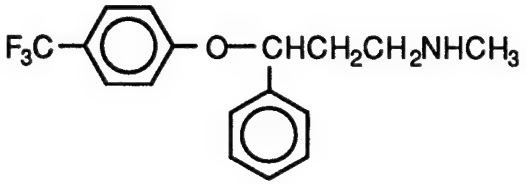
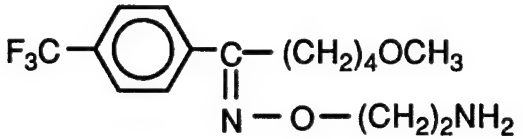
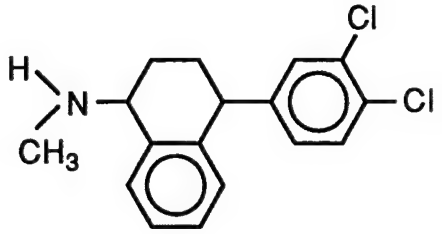
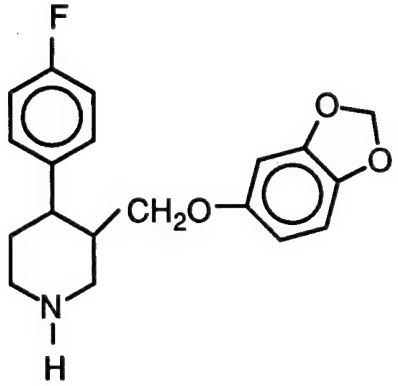
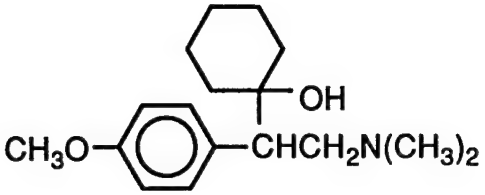
| Drug | Structure | $t_{1/2}$ (hr) | $V_d$ (L/kg) | Metabolite |
|---|---|---|---|---|
| trazodone |  | 4-7 | 0.9-1.5 | 1-(*m*-chlorophenyl)-piperazine |
| bupropion |  | 4-24 | 40 | threoamino alcohol, bupropion morpholinol metabolite |
| fluoxetine |  | 24-72 | 26 | norfluoxetine |
| fluvoxamine |  | 16-26 | 25 | fluvoxamine acid |

Table III.1 (Continued).

| Drug | Structure | $t_{1/2}$ (hr) | $V_d$ (L/kg) | Metabolite |
|---|---|---|---|---|
| sertraline |  | 24-26 | ? | *n*-desmethylsertraline |
| paroxetine |  | 7-37 | 3-28 | *n*-desmethylparoxetine* |
| venlafaxine |  | 3-7 | 4-12 | *o*-desmethylvenlafaxine |

* *n*-desmethylparoxetine is the only active metabolite among the drugs studied.

## 3.2. Materials and Methods.

### 3.2.1. Standards, reagents and solvents.

Trazodone (Desyrel) was obtained from Sigma, *m*-chlorophenyl-piperazine (mCPP) was a gift from Bristol-Myers Squibb, sertraline (Zoloft) and *n*-desmethylsertraline were gifts from Pfizer Inc., paroxetine (Paxil) was a gift from SmithKline Beecham, fluoxetine (Prozac) and norfluoxetine were obtained from Eli Lilly & Co., bupropion (Wellbutrin), threoamino alcohol, and the morpholinol metabolite were obtained from Burroughs Wellcome, Inc., and venlafaxine (Effexor) and *o*-desmethylvenlafaxine were gifts from Wyeth-Ayerst. All other reagents were analytical grade or better and were obtained from Fisher.

### 3.2.2. Gas chromatography.

Gas chromatography/mass spectrometry (GC/MS) was performed on a 5890/5970 GC/MS from Hewlett Packard (Palo Alto, CA). Chromatographic separation was achieved using a 5% phenylmethylsilicone column (30 m x 0.32-micron i.d., Econocap; Alltech). Analyses were initially performed using the temperature program discussed in Chapter 2. However, bupropion's morpholinol metabolite, norfluoxetine, fluoxetine, and fluvoxamine were not resolved from each other under these conditions. Also, quantitation could not be performed below 0.1 mg/L using this method. To maximize resolution, a temperature program from 120 to 200°C at 10°C/min, 200 to 220°C at 4°C/min, and 220 to 295°C at 8°C /min, held at the final temperature for 10 minutes was

used. To increase sensitivity, analyses were performed using Selected Ion Monitoring (SIM) mode, in which the detector counts only specified ions of interest, thereby decreasing background. The ions monitored using this method are highlighted in boldface in Table III.2.

Table III.2. Principal Ions in the Mass Spectra of Serotonergic Drugs and Metabolites.

| Compound | Principal ions (m/z) |
|---|---|
| bupropion | **100**, 57, 111, 75, **224**, 139, 166, 226 |
| threoamino alcohol | **100**, 77, 57, **208**, 115, 139, 226, 166 |
| bupropion morpholinol metabolite | 44, **116**, 139, 111, 84, **224**, 226, 166 |
| norfluoxetine | 134, **104**, 191, 77, 162, 51, 132, 251 |
| fluoxetine | **309**, 59, **104**, 78, 148, 115, 183, 164 |
| fluvoxamine | **187**, 71, **276**, 45, 172, 200, 145, 299 |
| *n*-desmethylsertraline | **119**, **274**, 246, 292, 104, 193, 228, 159 |
| sertraline | **274**, **159**, 262, 132, 103, 239, 304, 202 |
| trazadone | **205**, 70, 176, 56, 138, 231, 278, 166, **371** |
| *o*-desmethylvenlafaxine | **58**, **120**, 165, 91, 77, 149, 188, 213 |
| venlafaxine | **58**, **134**, 179, 91, 121, 81, 180, 203 |
| paroxetine | **329**, **192**, 70, 138, 177, 109, 53, 123 |

### 3.2.3. High performance liquid chromatography.

For high performance liquid chromatographic (HPLC) analysis, a reversed-phase isocratic method with photodiode array detection

(PDA) was used. The system setup was the same as that for tramadol analysis [a Gilson 305/307 pumping unit (Gilson, Middleton, WI), a Rheodyne sample injector (Model 7161, Rheodyne) equipped with a 20 μL loop, a 1040M photodiode array detector (Hewlett Packard), and a Lichrosorb RP Select B column (5 μm particle size, 250 mm x 4.6 mm I.D., Merck; Alltech). A flow rate of 1.5 ml/min was used, and data were collected in peak capture mode from 190 to 400 nm, with pilot wavelengths at 260 nm/230 nm.

An experimental design similar to that discussed in Chapter 2 for tramadol analysis was used, with the exception that only mobile phase combinations with the RP Select B column were investigated. The bonded phase in this column is specially treated to minimize peak tailing associated with interaction with basic compounds, and the packing is irregularly shaped to enable greater separation. A mix of SSRI's and metabolites were analyzed using mobile phase combinations of 10, 20, 40, and 50% acetonitrile in 0.05M phosphate buffer (pH 3). The mobile phase which enabled the greatest resolution and provided the best peak shape contained 40% acetonitrile.

### 3.2.4. Case Samples.

Blood samples collected at autopsy during the investigation of fifty-two unrelated fatalities were each placed in separate 10-ml vials containing sodium fluoride and potassium oxalate (Vacutainer; Becton Dickinson, New Jersey). The samples were

refrigerated until analysis was performed. Most samples were from peripheral blood, but some were from central blood, and some were not labeled as to which type of blood they came from (see Tables III.5-III.10). This distinction is made because postmortem peripheral and central blood levels can vary a great deal, especially for drugs with a volume of distribution ($V_d$) greater than 3 L/kg. In a study of heart and femoral blood concentrations of a variety of drugs (21), Prouty and Anderson found that in heart blood, the concentration of each drug increased as the postmortem interval increased, and that femoral blood concentrations more closely approximated concentrations in field blood (blood taken via cardiac puncture by a medical examiner during the course of a death investigation).

### 3.2.5. Sample preparation.

All drugs and metabolites were extractable using the same *n*-butyl chloride procedure discussed in Chapter 2 (69, 70). However, yields for the metabolites were generally lower than those of the parent drugs, presumably due to their greater polarity. Analysis of samples using the SIM method compensated for losses in extraction by lowering the limit of detection (LOD) and limit of quantitation (LOQ). To recap, blood (1 ml), internal standards (diphenylamine and metycaine, 100 ul of 1-mg/L and .5 mg/L solutions, respectively), and pH 9 saturated potassium borate buffer (1 ml) were mixed and extracted with *n*-butyl chloride (3 ml). The organic fraction was back extracted into 3M hydrochloric

acid (200 ul), which was then basified with concentrated ammonium hydroxide and reextracted into chloroform (100 ul). A two microliter aliquot of the resulting extract was then injected for analysis by GC/MS, and the remaining chloroform was evaporated to dryness under air at 20°C.

**3.3. Results.**

Figure III.1 is a standard mass chromatogram showing resolution of bupropion, fluoxetine, fluvoxamine, sertraline, venlafaxine, paroxetine, and trazodone from their metabolites and both internal standards, metycaine and diphenylamine. Bupropion's morpholinol metabolite and norfluoxetine were not completely resolved using this method, but there were no cases where both bupropion and fluoxetine were present. To enable quantitation, norfluoxetine ions were not monitored when analyzing cases with bupropion, and morpholinol metabolite ions were not monitored when analyzing fluoxetine cases. To permit detection of these compounds in the same run, however, a modified temperature program (120 to 180°C at 15°C/min, then 180 to 295°C at 8°C/min, holding the final temperature for 8 minutes) could be used, completely resolving the two compounds.

The principal ions for the mass spectra for each drug and metabolite are listed in Table III.2. Trazodone's active metabolite, *m*-chlorophenyl-piperazine (mCPP), was not detectable by GC/MS without derivatization, due to its polarity. Previously published methods for trazodone determination involved derivatization (100,

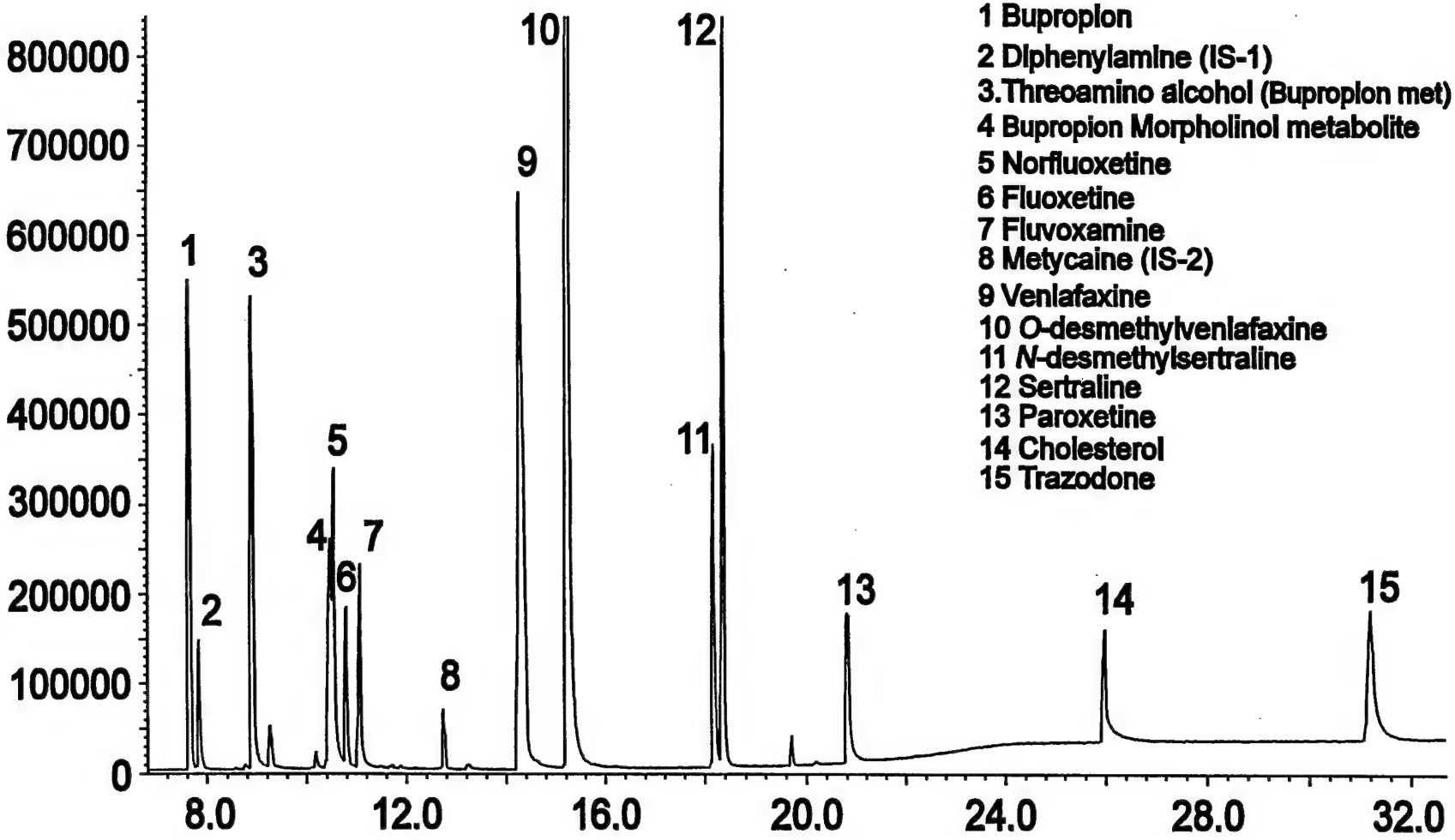



Figure III.1. Separation of atypical antidepressants and metabolites.

101).

Figure III.2 is a liquid chromatogram showing separation of venlafaxine, paroxetine, *n*-desmethylsertraline, and sertraline. The HPLC was not used for quantitation of drug in the case samples because of interference with peaks of interest from the early eluting compounds in a biological matrix. The method would have been especially useful for quantitation of mCPP since it could not be detected with GC/MS, but accurate quantitation of this metabolite could not be achieved because it was not completely resolved from trazodone. The method was useful for qualitative comparison of drug levels, though, as it has been recognized that substantial concentrations of mCPP appear at higher doses of the parent drug (13).

The GC/MS method was, however, suitable for quantitation of all drugs and other metabolites, and was linear over the range 0.01-10.00 mg/L. The limits of detection (LOD) and quantitation (LOQ), determined as explained in Chapter 2, i.e. 3 x $SD_o$ for LOD and 5 x $SD_o$ for LOQ, were uniform and were 0.01 and 0.05 mg/L, respectively. Figure III.3 shows standard deviation as a function of venlafaxine concentration used for determining LOD and LOQ. Regression coefficients for each drug were 0.994 (bupropion), 0.997 (threoamino alcohol), 1.000 (bupropion morpholinol metabolite), 0.999 (fluoxetine), 0.997 (norfluoxetine), 0.999 (sertraline), 0.998 (*n*-desmethyl-sertraline), 0.997 (venlafaxine), 0.986 (*o*-desmethylvenlafaxine), and 0.999 (trazodone).

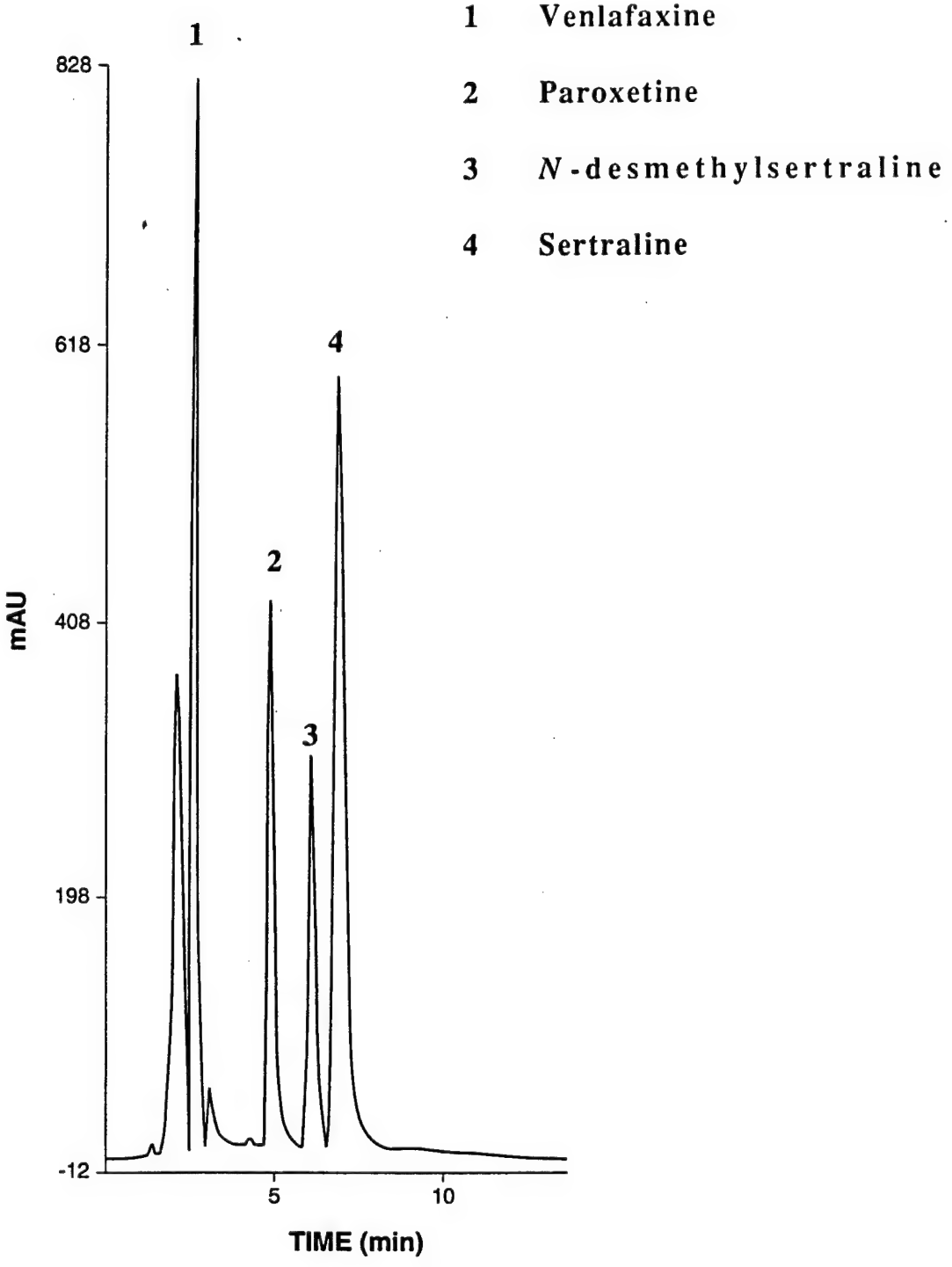



Figure III.2. Liquid chromatographic separation of venlafaxine, paroxetine, *n*-desmethylsertraline, and sertraline.

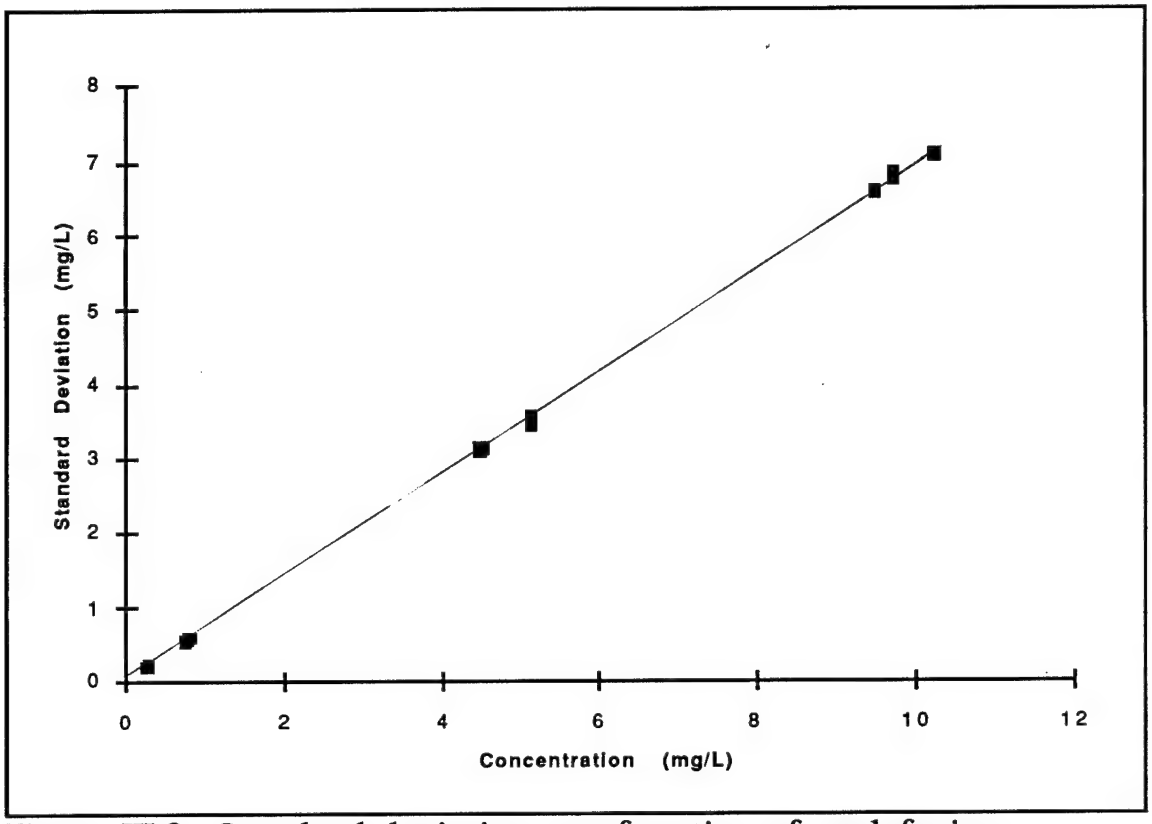



Figure III.3. Standard deviation as a function of venlafaxine concentration.

Therapeutic and toxic ranges for drugs present in the blood samples are summarized in Table III.3, in addition to their relative ability to inhibit neurotransmitter reuptake and the CYP450 isozyme(s) (if known) for which they are substrates (1, 14, 22, 23). Concentrations of each drug and metabolite and other drugs found in each of fifty-two fatalities, along with manner and cause of death are included in Tables III.5-III.10. Circumstances surrounding each death are also included. Table III.4 lists drug and metabolite concentrations found in postmortem tissue samples

Table III.3. Properties of Drugs Found in Postmortem Blood.

| Drug | Therapeutic conc, mg/L* | Toxic conc, mg/L* | Reuptake Inhibition NE | DA | ST |
|---|---|---|---|---|---|
| acetominophen (2E1) | 4.20-52.00 | 30.00-300.00 | 0 | 0 | 0 |
| alprazolam (2D6, 3A4) | 0.02-0.04 | 0.069 | 0 | 0 | 0 |
| amitriptyline (2D6, 3A4) | 0.06-0.22 | >1.00 | ± | 0 | ++ |
| amphetamine | 0.10-3.00 | 0.20-3.00 | ++ | + | + |
| bupropion† | 0.01-0.39 | 4.00-14.00 | + | ++ | 0 |
| butalbital (3A4) | 1.00-10.00 | 10.00-25.00 | 0 | 0 | 0 |
| carbamazepine (3A4, induces 1A2) | 4.00-8.00 | 10.00-120.00 | 0 | 0 | 0 |
| chlordiazepoxide | 0.50-2.30 | 1.00-66.00 | 0 | 0 | 0 |
| cocaine | 0.80±0.20 | 0.10-211.00 | 0 | 0 | 0 |
| codeine (2D6-to morphine) | 0.03-0.34 | 1.00-8.80 | 0 | 0 | 0 |
| cyclobenzaprine | 0.003-0.034 | >0.46 | 0 | 0 | ± |
| dextromethorphan (2D6, 3A4)) | 0.38 | LD = 0.5 g | 0 | 0 | + |
| diazepam (2C19) | 0.10-2.50 | >1.50 | 0 | 0 | 0 |
| diltiazem (3A4, inhibits 1A2, 2D6, 3A4) | 0.10-0.20 | 1.70-33.00 | 0 | 0 | 0 |
| diphenhydramine | 0.014-0.112 | 0.10-31.00 | 0 | 0 | 0 |
| doxepin | 0.03-0.15 | >0.10 | ± | 0 | ± |
| fentanyl | 0.03-0.38 | 0.30-3.90 | 0 | 0 | 0 |

Table III.3 (Continued).

| Drug | Therapeutic conc, mg/L* | Toxic conc, mg/L* | Reuptake Inhibition NE | DA | ST |
|---|---|---|---|---|---|
| fluoxetine (2D6, 3A4) | 0.06-0.453 | 1.30-6.80 | 0 | 0 | +++ |
| fluvoxamine (inhibits 1A2, 2D6, 3A4) | 0.02-0.42 | > 0.42 | 0 | 0 | + |
| hydrocodone (2D6) | 0.002-0.024 | 0.13-7.00 | 0 | 0 | 0 |
| hydroxyzine | 0.031-0.07 | 1.10-103.00 | 0 | 0 | 0 |
| ibuprofen | 17.00-49.00 | 84.00-700.00 | 0 | 0 | 0 |
| lidocaine (3A4) | 1.90-5.00 | 6.00-33.00 | 0 | 0 | 0 |
| loxapine | 0.017 | 0.19-7.70 | ++ | + | 0 |
| meperidine | 0.16-0.52 | 1.00-20.00 | 0 | 0 | 0 |
| meprobamate | 6,40.00-27.00 | 60.00-240.00 | 0 | 0 | 0 |
| methadone | 0.28-1.06 | 0.06-1.80 | 0 | 0 | 0 |
| methamphetamine | 0.01-0.02 | 0.15-40.00 | ++ | + | 0 |
| morphine | 0.01-0.07 | 0.12-4.70 | 0 | 0 | 0 |
| nortriptyline (2D6) | 0.01-0.375 | >1.00 | ++ | 0 | ± |
| oxycodone (2D6) | 0.009-0.038 | 0.40-14.00 | 0 | 0 | 0 |
| paroxetine (2D6, inhibits 2D6, 3A4) | 0.031-0.062 | ≥0.24 | 0 | 0 | + |
| phenytoin (2C19, induces 1A2, 3A4) | 10.00-20.00 | 16.00-112.00 | 0 | 0 | 0 |
| promethazine | 0.006-0.099 | 2.40-12.00 | 0 | + | 0 |

Table III.3 (Continued).

| Drug | Therapeutic conc, mg/L* | Toxic conc, mg/L* | Reuptake Inhibition NE | DA | ST |
|---|---|---|---|---|---|
| propoxyphene (2D6) | 0.05-0.75 | 1.00-2.00 | + | 0 | + |
| sertraline (2D6, 3A4) | 0.03-0.19 | 0.25-0.61 | 0 | 0 | + |
| temazepam | 0.21-1.10 | 0.90-14.00 | 0 | + | 0 |
| thioridazine (2D6) | 0.40-2.00 | >2.00 | 0 | + | 0 |
| trazodone (3A4)ǂ | 0.49-1.60 | >15.00 | 0 | 0 | + |
| venlafaxine (2D6, 3A4) | 0.07 | >0.245 | + | 0 | ++ |
| verapamil (3A4) | 0.055-0.355 | 0.90-85.00 | 0 | 0 | 0 |

Abbreviations and symbols: NE = norepinephrine; DA = dopamine; ST = serotonin; + to ++ = active to strongly active; ± = weakly active; 0 = lacking

* Levels are taken from Baselt (22) except fluvoxamine, which came from USPDI update (23).

† Bupropion's active metabolite is a suspected CYP2D6 substrate (116).

ǂ Trazodone's congener, nefazodone, has been shown to be a substrate for CYP3A4, so trazadone is likely also a substrate for this isoenzyme (4).

analyzed in eight of the cases.

### 3.4. Discussion.

With the exception of paroxetine, all of the atypical antidepressants and many TCA's have active metabolites. The active metabolites of the TCA's strongly inhibit norepinephrine reuptake, whereas the those of the SSRI's are selective for serotonin reuptake inhibition like their parent drugs. The serotonin reuptake-inhibiting ability of SSRI active metabolites

## Table III.4. Postmortem Tissue Distribution of Atypical Antidepressants*.

| # | Age | Sex | Central Blood (mg/L) | Peripheral Blood (mg/L) | Bile (mg/L) | Spleen squeeze (mg/L) | Urine (mg/L) | Liver (mg/kg) | Gastric Contents (mg/kg) | Vitreous (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Trazodone** | | | | | | | | | | |
| 1 | 51 | M | 0.08 | 0.06 | 0.06 | – | 0.42 | 1.25 | 0.87 | 0.05 |
| 6 | 44 | F | 0.39 | 0.25 | 0.42 | – | 0.67 | – | 0.57 | 0.41 |
| 7 | 44 | F | 0.65 | 0.35 | 1.02 | – | 4.52 | 1.90 | 38.12 | 0.08 |
| 13 | 36 | F | 11.45 | 9.06 | 12.92 | – | 28.29 | 3.52 | 308.84 | 4.70 |
| 16 | 41 | M | – | – | – | 2.65 | – | – | – | – |
| **Sertraline & n-desmethylsertraline** | | | | | | | | | | |
| 20 | 44 | F | 1.79 | 2.52 | 3.08 | – | 5.65 | 42.14 | 8.72 | 0.89 |
| | | | 1.95 | 4.05 | 3.95 | – | 9.21 | 59.69 | 19.91 | 1.46 |
| **Fluoxetine & Norfluoxetine** | | | | | | | | | | |
| 15 | 41 | M | – | – | – | 6.66 | – | – | – | – |
| | | | – | – | – | 20.27 | – | – | – | – |
| **Paroxetine** | | | | | | | | | | |
| 1 | 39 | M | 1.41 | 0.20 | 4.27 | – | 9.49 | 0.70 | 0.16 | <0.01 |
| **Venlafaxine & *o*-desmethylvenlafaxine** | | | | | | | | | | |
| 2 | 47 | F | 0.50 | 0.75 | 0.68 | – | 0.55 | 0.87 | 3.17 | 1.04 |
| | | | 0.51 | 0.28 | 2.29 | – | 5.58 | 1.60 | 2.26 | 1.51 |
| 3 | 50 | F | 1.34 | 0.97 | 2.14 | – | 2.16 | 1.88 | 1.82 | 0.62 |
| | | | 1.35 | 1.03 | 2.74 | – | 7.77 | 2.07 | 3.24 | 0.96 |

* Metabolite concentrations listed below those of parent drugs.

may be involved with a synergistic effect between SSRI's, their active metabolites, and other serotonergic drugs present. For these reasons, it is important to consider metabolite concentrations when interpreting parent drug levels.

The half-lives of most atypical antidepressants range from a few hours to several days (see Table III.1). The longer half-lives are potentiated by the fact that most SSRI's exhibit autoinhibition (inhibition of a drug's metabolism by itself) (14).

As was mentioned earlier, many serotonergic drugs are substrates for CYP2D6 and 3A4, and as such are subject to metabolic inhibition, which may affect therapeutic response and increase concentrations above levels associated with given doses. The presence of other drugs which may interfere with isoenzyme metabolism must therefore be considered in cases involving serotonergic drugs. The possibility of metabolic inhibition makes it important to consider metabolite concentrations when interpreting parent drug levels.

The role of serotonergic drugs in fatalities is often difficult to determine because of the number of mechanisms of toxicity associated with them. An investigation of the circumstances surrounding each case in this study will provide investigators with baseline information regarding blood levels associated with different types of fatalities. No antemortem medical evaluation was performed on any of the subjects described here, so consideration of the role of SS in these fatalities was based solely

on the degree of serotonergic activity of all drugs present.

### 3.4.1. Trazodone.

In clinical studies involving four adult subjects, an average peak plasma concentration of 2.1 mg/L of trazodone and 0.01 mg/L of mCPP, the major metabolite of trazodone, were measured within 2-4 hours after being given a single 150 mg oral dose (100). Steady-state plasma levels of trazodone ranged from 0.49-1.21 mg/L in five patients treated for depression, while concentrations of mCPP ranged from 0.01-0.03 mg/L (101). While mCPP is the only active metabolite of trazodone, some of the dose is converted to beta-(3-oxo-s-triazolic(4-3α)pyridin-2-yl)propionic acid (OTPA), with the rest as glucuronidated metabolites. Despite the fact that trazodone has been widely used since its entry onto the market, questions continue to be raised regarding efficacy and toxicity related to its use. Shopsin *et al.* (24) cite several unpublished double-blind controlled studies conducted by different groups in which trazodone-treated patients had extremely low rates of response (10-20%).

Consequently, a problem associated with trazodone therapy is the inability of some patients to tolerate doses sufficient to provide the antidepressant effects of the drug due to the onset of side effects such as oversedation, light-headedness, or confusion. Male priapism and mania are two other common non-toxic side effects associated with trazodone. Orthostatic hypotension is a predictable but serious consequence of trazodone's potent inhibition of α

receptors. A series of case reports (102-104) has shown that trazodone can induce or aggravate arterial and ventricular arrhythmia, particularly in patients with pre-existing heart disease, but even in individuals with healthy cardiovascular function.

Trazodone appears to be safer in overdose than most other antidepressants, causing only mild central nervous system depression (although possibly serious hypotension) and few fatalities even in large overdoses of trazodone alone (105-110). In addition to these side effects, drug interactions associated with combined serotonergic activity and/or competitive inhibition of CYP3A4 due to the presence of other serotonergic drugs or 3A4 substrates or inhibitors may contribute to increased blood trazodone levels and subsequent associated toxicity in trazodone-related fatalities. Although the isoenzyme responsible for trazodone metabolism is unknown, its congener, nefazodone, is a substrate for 3A4.

There has been one case of Serotonin Syndrome (SS) reported in the literature involving trazodone and buspirone, a specific 5-$HT_{1\bar{A}}$ agonist, in which only myoclonus appeared in the subject (80). Reports of trazodone-related fatalities in the literature are rare. Two such studies, both involving women in their 40's who were found dead at their residences, had blood trazodone levels of 15 and 23 mg/L, respectively (81, 82).

Table III.5 contains information regarding the trazodone-related

## Table III.5. Case Information on Decedents Testing Positive for Trazodone.

| # | Age | Sex | Traz (mg/L) | Alcohol (g/100 mL) | Other Drug Use (mg/L) | Circumstances Surrounding Death | Cause of Death | Manner of Death | Central or Peripheral Blood? |
|---|---|---|---|---|---|---|---|---|---|
| Blood | | | | | | | | | |
| 1 | 51 | M | 0.06 | neg | cocaine metabolite* lidocaine | Hx of extensive heart trouble; subject stood suddenly from couch & collapsed on floor; had complained of heartburn & side pains; on potassium chloride, isosorbide, & furosemide Rx, but noncompliant w/ meds | atherosclerotic cardiovascular disease w/ severe coronary atherosclerosis & a healing myocardial infarct | natural | peripheral |
| 2 | 42 | M | 0.11 | neg | methadone (0.18) valproic acid (16.00) | Hx of severer obesity, depression w/ bipolar disorder, sleep apnea, chronic pain, ASHD | acute myocardial infarction due to atherosclerotic heart disease | natural | unknown |
| 3 | 50 | M | 0.11 | neg | morphine (0.07) benzoylecgonine (2.20) meperidine (0.60) diltiazem (0.17) | known acute & chronic opiate and cocaine abuser | bacterial endocarditis w/ sepsis due to parenteral drug abuse | undetermined | peripheral |
| 4 | 38 | M | 0.13 | neg | propoxyphene (8.80) norpropoxyphene (8.20) diazepam (0.20) nordiazepam (0.42) | Hx of depression & Crohn's disease; multiple med. bottles found at scene | acute propoxyphene intoxication | accident | unknown |
| 5 | 40 | M | 0.21 | 0.14 | – | strangulation marks on neck from blanket, right carotid perforation due to stab wound to right side of neck | right carotid perforation due to stab wound to right side of neck | homicide | peipheral |

Table III.5 (Continued).

| # | Age | Sex | Traz (mg/L) | Alcohol (g/100 mL) | Other Drug Use (mg/L) | Circumstances Surrounding Death | Cause of Death | Manner of Death | Central or Peripheral Blood? |
|---|---|---|---|---|---|---|---|---|---|
| 6 | 44 | F | 0.25 | neg | morphine, periph. (0.20) morphine, central (0.27) | subject found dead at friend's residence | acute intoxication due to combined effects of opiates, trazodone | accident | peripheral |
| 7 | 44 | F | 0.35 | 0.18 | dextromethorphan (0.49) promethazine (0.98) antipyrine cocaine (<0.05) cocaethylene (<0.05) benzoylecgonine (0.57) sertraline (2.52) *n*-desmethylsertraline (4.05) | Hx of chronic alcoholism; autopsy findings: pill fragments in stomach, nodular cirrhosis w/ splenomegaly | acute combined drug intoxication; contributory cause: advanced micronodular cirrhosis w/ prominent splenomegaly | accident | peripheral |
| 8 | 61 | F | 0.37 | neg | -- | Hx of coronary artery disease; subject died at home | coronary artery atherosclerosis w/ thrombosis | natural | peripheral |
| 9 | 43 | M | 0.40 | neg | alprazolam (<0.1) fluoxetine (0.24) norfluoxetine (0.52) | Hx of depression, Rx include Paxil, alprazolam, trazodone, Vicodin, ephedrine; autopsy findings: gunshot wound to head | penetrating gunshot wound to head | sucide | unknown |
| 10 | 38 | M | 0.50 | neg | diphenhydramine (1.20) loxapine (1.40) sertraline (0.75) *n*-desmethylsertraline (0.38) | hit in county jail; wrote suicide note to wife & took apparent drug overdose; resuscitation attempts unsuccessful | multiple drug intoxication | suicide | peripheral |

Table III.5 (Continued).

| # | Age | Sex | Traz (mg/L) | Alcohol (g/100 mL) | Other Drug Use (mg/L) | Circumstances Surrounding Death | Cause of Death | Manner of Death | Central or Peripheral Blood? |
|---|---|---|---|---|---|---|---|---|---|
| 11 | 35 | F | 0.99 | neg | methadone (1.20) promethazine (0.81) hydroxyzine (0.33) fluoxetine (0.66) norfluoxetine (0.54) | subject was taking multiple pain meds; pulmonary edema found at autopsy | pulmonary embolism, possibly complicated by incompitent mitral valve | natural | central |
| 12 | 39 | F | 1.41 | 0.19 | propoxyphene (6.60) norpropxyphene (0.87) codeine (7.00) acetominophen (521.00) ibuprofen (37.00) hydrocodone (1.28) diphenydramine (1.09) sertraline (1.76) *n*-desmethylsertraline (0.15) | subject found dead in motor vehicle; identified as official missing person as of 2 days prior | acute intoxication due to combined effects of acetominophen, codeine, propoxyphene, hydrocodone, trazodone, sertraline, ibuprofen, diphenhydramine, & ethanol | probable suicide | unknown |
| 13 | 36 | F | 9.06† | 0.05 | oxycodone (0.7) acetominophen (35.7) | subject found dead at home; Hx of alcohol abuse, depression, & suicide ideation, suicide note found; on Desyrel Rx, also possessed mother's meds: percocet, diphenhydramine, Zoloft, & Ativan | acute intoxication due to combined effects of trazodone, acetominophen, oxycodone & ethanol | suicide | peripheral |

## Table III.5 (Continued).

| # | Age | Sex | Traz (mg/L) | Alcohol (g/100 mL) | Other Drug Use (mg/L) | Circumstances Surrounding Death | Cause of Death | Manner of Death | Central or Peripheral Blood? |
|---|---|---|---|---|---|---|---|---|---|
| 14 | 45 | F | 24.32† | neg | diltiazem (3.35) temazepam (7.75) sertraline (1.76) *n*-desmethylsertraline (0.57) | Hx of depression w/ apparent multiple drug overdose | acute intoxication by combined effects of trazodone & benzodiazepines | suicide | peripheral |
| 15 | 32 | F | 32.91† | 0.22 | fluoxetine (1.19) norfluoxetine (0.83) | found dead in motel room w/ multiple empty Rx drug bottles (trazodone, lorazepam, valproic acid, Prozac), no suicide note found | acute alcohol & Rx drug overdose | accident | unknown |
| Spleen | | | | | | | | | |
| 16 | 41 | M | 2.65† | 0.05 | fluoxetine (6.66) norfluoxetine (20.27) | friends had not seen decedent recently & became alarmed at foul smell pervading hallway outside his apartment; found dead, body in advanced decomposition | arteriosclerotic cardiovascular disease | natural | N/A |

* Not quantitated due to interference from lidocaine.
† Concentration above therapeutic range.

deaths analyzed in this study. Many of these fatalities (Cases 4, 10, 12, 14-16) were attributed to acute drug intoxication, although only Cases 14 and 15 were clearly caused by acute trazodone overdose. Central nervous system depression caused by alcohol may have played a role in Case 15. Isoenzyme metabolism may have been a factor in Cases 1, 10, 11, and 14-16; fluoxetine, norfluoxetine, sertraline, and diltiazem are inhibitors of CYP3A4, and sertraline, diltiazem, and lidocaine are 3A4 substrates. The serotonergic activity of several drugs present probably also contributed to fatality in Cases 4, 7, 9-12, and 14-16.

The presence of cardiovascular disease might have been a predisposing factor in Cases 1, 2, 8, 11, and 16. Although the trazodone level in Case 1 was fairly low, the subject's healing myocardial infarct might have been exacerbated by a metabolic interaction between trazodone and lidocaine due to trazodone's association with repetitive ventricular arrhythmias and atrial tachycardia.

Postmortem distribution of trazodone was examined in Cases 1, 6, 10, and 13 (Table III.4). In all cases, the central blood trazodone levels were higher than those of peripheral blood. This is consistent with the only other report comparing trazodone levels in these specimens (21). Urine and liver levels were the highest of all of the specimens in all four cases, with the exception of gastric contents. Levels of drug in gastric contents are difficult to interpret due to the inhomogeneity of this specimen. Thus, the

high concentration of trazodone in Case 13 is more likely due to the presence of pill fragments in the sample, although concentration of trazodone in the other tissues were higher in this case than in any others. The concentration of drug in the spleen in Case 16 is difficult to interpret without having other tissue samples to analyze. However, all drug levels found were above therapeutic blood levels (22).

### 3.4.2. Bupropion.

Clinical trials revealed serum levels of 0.01-0.39 mg/L after single dosing at 20-200 mg of oral bupropion. Peak plasma concentrations of the two major metabolites, threoamino alcohol and the morpholinol metabolite, ranged from 0.03-0.21 and 0.09-0.49 mg/L, respectively (111-113). These two compounds are believed to possess amphetamine-like activity which may inhibit both norepinephrine and dopamine reuptake (1), and have somewhat longer half-lives than the parent drug, resulting in plasma metabolite concentrations which exceed those of bupropion.

Bupropion was withdrawn from the research and new humanitarian-use market just as it was about to be released in March of 1986 (13) due to the occurrence of generalized seizures in 4 of 69 non-depressed bulimic patients at 300-325 mg/day divided doses in a multicenter clinical trial (114). However, at least some cases of bupropion-associated seizures proved to involve other risk factors for lowered seizure threshold. No significant orthostatic hypotension has been demonstrated with

bupropion, even in cases of pre-existing heart disease (115).

Bupropion appears to be relatively safe on overdose, although fatalities can occur at concentrations beyond 10 times the therapeutic range. In deaths from other causes where bupropion is present, concentrations of up to 7 times the therapeutic range may be encountered. Even though metabolite concentrations in plasma tend to exceed those of bupropion, toxicity in overdose does not appear to be related to threoamino alcohol concentrations (83).

Like trazodone, bupropion may be subject to metabolic inhibition, which may play a role in deaths associated with the drug. In a study of the metabolism of bupropion (116), the authors hypothesized that while bupropion does not appear to inhibit or be metabolized by CYP2D6, this isozyme may be involved in the biotransformation of the morpholinol metabolite, as plasma level/dose ratios of this compound were significantly higher in poor 2D6 metabolizers than in normal subjects. Therefore, the presence of other drugs which inhibit or are substrates for 2D6 may contribute to increased plasma bupropion levels, thereby enhancing bupropion's pharmacological effect. Additionally, although bupropion is not directly associated with blockade of serotonin reuptake, it may act to augment the serotonergic activity of other drugs present, which may contribute to toxicity due to interaction with dopamine and 5-$HT_2$-receptors (1).

Several cases of bupropion-related deaths have been reported in

the literature, five of which occurred in adults who ingested from 4-15 g of the drug (84-86). Corresponding bupropion levels in these cases ranged from 4.2-13.2 mg/L. In five unrelated overdoses involving bupropion reported by this laboratory, blood levels ranged from 4.0-20.0 mg/L of bupropion, 3.4-12.8 mg/L of the morpholinol metabolite, and 10.4-22.0 mg/L of threoamino alcohol (83).

Table III.6 lists postmortem cases involving bupropion. Combined bupropion and cocaine intoxication seems to be the only plausible explanation for Case 4. Case 5 is a clear case of bupropion overdose, although the presence of *n*-desmethylsertraline might have contributed to toxicity due to the combined effect of the contracting action of extra circulating serotonin and vasoconstriction due to the subject's heart condition.

**3.4.3. Fluoxetine.**

A single, oral 40 mg dose in adults has been shown to produce peak plasma fluoxetine levels of 0.02-0.06 mg/L within 6-8 hours (117). In multiple-dose clinical trials, 24 patients receiving 20-60 mg of the drug daily developed steady-state plasma levels of 0.03-0.47 mg/L of fluoxetine and 0.02-0.47 mg/L of norfluoxetine (78).

Norfluoxetine, fluoxetine's active metabolite, is also specific for 5-HT reuptake inhibition, and both compounds exhibit autoinhibition

## Table III.6. Case Information on Subjects Testing Positive for Bupropion.

| # | Age | Sex | Bup (mg/L) | TAA (mg/L) | BMM (mg/L) | Alcohol (g/100 mL) | Other Drug Use (mg/L) | Circumstances Surrounding Death | Cause of Death | Manner of Death | Central or Peripheral Blood? |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 34 | M | -- | 0.06 | 0.107 | neg | -- | subject found w/ penetrating rifle wound to head | penetrating rifle wound to head | suicide | unknown |
| 2 | 76 | M | 0.043 | 1.60 | 0.37 | neg | -- | subject found w/ contact gunshot wound to head | gunshot wound to head | suicide | unknown |
| 3 | 25 | F | 0.78* | 5.62 | 1.45 | neg | morphine (0.31) amitriptyline (0.88) nortriptyline (0.25) carbamazepine (32.8) | Hx of AIDS & depression; subject died suddenly; autopsy findings incl. pill fragments in stomach | acute multiple drug intoxication | suicide | unknown |
| 4 | 36 | M | 1.46* | 5.82 | 0.43 | neg | cocaine (<0.05) benzoylecgonine (1.04) glucose (250 mg/dL) | autopsy findings include Diabetes Mellitus and pill fragments in stomach | acute intoxication by combined effects of bupropion and cocaine | suicide | peripheral |
| 5 | 35 | F | 7.86* | 6.77 | 0.93 | neg | *n*-desmethylsertraline (1.09) | depression Hx & suicide attempts, arrested in ER after taking pills, unable to be resuscitated; incidental findings: coronary atheroscl., cardiomegaly, thyroid follicular adenoma | cardiorespiratory arrest 2° to drug overdose | not listed | unknown |

* Concentration above therapeutic range.

causing a non-linear relationship between dose and concentration. The most common side effects associated with fluoxetine therapy are bleeding disorders, Syndrome of Inappropriate Secretion of Antidiuretic Hormone (SIADH), and SS (14, 121). Clotting problems due to bleeding disorders may have serious consequences in patients with heart disease, due to vasoconstriction caused by the contracting action of platelet 5-HT. The appearance of SS is the most common life threatening side effect of pure fluoxetine overdose, and SS symptoms can be aggravated by the exhibition of autoinhibition by fluoxetine and norfluoxetine. However, SS can also present in cases where multiple drugs with serotonergic activity in addition to fluoxetine are measured at therapeutic concentrations. As with trazodone and bupropion, isoenzyme inhibition or induction can contribute to decreased fluoxetine clearance which may lead to toxic side effects in certain cases due to the presence of other substrates and/or inhibitors of CYP2D6 or 3A4, as both fluoxetine and norfluoxetine are substrates for CYP2D6 and inhibitors of 2D6 and 3A4.

Reports of SS involving fluoxetine, only some of which had fatal outcomes, have been reported in the literature, most commonly in conjunction with monoamine oxidase inhibitors (MAOI's) or L-Tryptophan (119, 120). Six fatalities reported in the literature in adults who ingested from 1-2 g of fluoxetine and, in four of the cases, at least one other drug, had blood fluoxetine and norfluoxetine levels ranging from 1.3-6.8 and 0.9-5.0 mg/L,

respectively (88-91).

Five of the fluoxetine-related fatalities in this study (Table III.7, Cases 10, 11, 13-15) were a result of acute drug intoxication with fluoxetine likely playing a major role, and three were cardiac-related (Cases 6, 7 and 15). The slightly elevated fluoxetine levels in Cases 6 and 7 might have contributed to these deaths due to vasoconstriction caused by increased serum serotonin levels, and enzyme saturation due to competitive inhibition of CYP3A4 by sertraline in Case 7 and trazodone in Case 6 might have potentiated this effect.

Cases 13 and 14 were clearly caused by acute fluoxetine intoxication. Although many other drugs were found in the subject's purse in Case 13, only fluoxetine and norfluoxetine were detected. It has been suggested that a possible indicator of acute fluoxetine overdose is a blood fluoxetine to norfluoxetine ratio of 2:1 or greater (14). This is true in both cases. Although the fluoxetine level in Case 13 is not as high as that in Case 14, the subject's abnormal heart condition probably played a contributory role in combination with vasoconstriction due to excess platelet 5-HT. Competitive inhibition of CYP2D6 or 3A4 may have contributed to toxicity in Cases 1, 3, 9 and 10, due to the presence of butalbital in Case 3 (3A4 substrate), propoxyphene in Cases 1 and 9 (2D6 substrate), and amitriptyline and nortriptyline in Case 10 (2D6 and 3A4 substrates). The serotonergic activity of the drugs in these cases is also likely to have played an important role.

## Table III.7. Case Information on Decedents Testing Positive for Fluoxetine.

| # | Age | Sex | Fluox (mg/L) | Norflu (mg/L) | Alcohol (g/100 mL) | Other Drug Use (mg/L) | Circumstances Surrounding Death | Cause of Death | Manner of Death | Central or Peripheral Blood? |
|---|---|---|---|---|---|---|---|---|---|---|
| Blood | | | | | | | | | | |
| 1 | 35 | F | 0.11 | 0.59 | 0.20 | acetominophen (116.7) propoxyphene (2.87) norpropoxyphene (2.38) diphenhydramine (0.33) | subject found dead in bed approx. 1 1/2 hrs after last seen alive; Rx include Prozac, propoxyphene/NAP & APAP | acute intoxication w/ multiple meds including propoxyphene & ethanol | accident | unknown |
| 2 | 43 | M | 0.24 | 0.52 | neg | alprazolam (<0.1) trazodone (0.40) | Hx of depression, Rx include Paxil, alprazolam, trazodone, Vicodin, ephedrine; autopsy findings: gunshot wound to head | penetrating gunshot wound to head | suicide | unknown |
| 3 | 41 | F | 0.27 | 0.56 | neg | butalbital (4.91) | subject found dead w/ pills in church parking lot, was in the middle of writing suicide note | listed as "pending toxicology" | undetermined | peripheral |
| 4 | 41 | F | 0.37 | 0.34 | 0.09 | chlordiazepoxide (3.36) nordiazepam (0.05) | subject found dead w/ self-inflicted oral gunshot wound | pulpification of brain due to self-inflicted gunshot wound | suicide | unknown |
| 5 | 38 | F | 0.47* | 0.31 | 0.04 | amitriptyline (0.20) nortriptyline (0.20) | Hx of alcohol abuse, peptic ulcers, & seizure disorder; prior suicide attempts; found dead by medics upon arrival at scene; numerous bruises observed on neck | fatty metamorphosis of liver | natural | peripheral |
| 6 | 35 | F | 0.66* | 0.54 | neg | methadone (1.20) promethazine (0.81) hydroxyzine (0.33) trazodone (0.99) | subject was taking multiple pain meds; pulmonary edema found at autopsy | pulmonary embolism, possibly complicated by incompitent mitral valve | natural | central |

Table III.7 (Continued).

| # | Age | Sex | Fluox (mg/L) | Norflu (mg/L | Alcohol (g/100 mL) | Other Drug Use (mg/L) | Circumstances Surrounding Death | Cause of Death | Manner of Death | Central or Peripheral Blood? |
|---|---|---|---|---|---|---|---|---|---|---|
| 7 | 75 | M | 0.67* | 0.29 | 0.12 | amphetamine (2.10) sertraline (0.57) *n*-desmethylsertraline (0.55) | found diseased at home; body moderately decomposed when found | coronary artery atherosclerosis | natural | unknown |
| 8 | 31 | F | 0.68* | 0.38 | neg | amitriptyline (0.10) nortriptyline (0.40) cyclobenzaprine (0.20) phenytoin (2.50) morphine (0.04) | found dead in bed; Hx of insulin dependent Diabetes, seizure disorder, renal failure, peripheral neuropathy, retinopathy, chronic pain; Rx include morphine, clonapin, amitriptyline, Dilantin, Prozac, cyclobenzaprine, insulin NPH | diabetes mellitus | natural | unknown |
| 9 | 50 | F | 0.69* | 0.35 | neg | acetominophen (22.20) propxyphene (0.33) norpropoxyphene (0.95) morphine (periph) (0.07) morphine (cent) (0.06) | subject found dead at residence | acute combined drug intoxication | accident | unknown |
| 10 | 48 | F | 1.03* | 0.40 | 0.04 | fentanyl (0.004) amitriptyline (0.89) nortriptyline (0.75) | Hx of mental illness, drug overdoses, & migraine headaches; subject died suddenly; Rx include amitriptyline, fluoxetine, tramadol, klonopin | acute multiple drug intoxication | accident | unknown |
| 11 | 32 | F | 1.19* | 0.83 | 0.22 | trazodone (32.91) | found dead in motel room w/ empty drug bottles (trazodone, lorazepam, valproic acid, Prozac), no suicide note found | acute alcohol & Rx drug overdose | accident | unknown |

Table III.7 (Continued).

| # | Age | Sex | Fluox (mg/L) | Norflu (mg/L | Alcohol (g/100 mL) | Other Drug Use (mg/L) | Circumstances Surrounding Death | Cause of Death | Manner of Death | Central or Peripheral Blood? |
|---|---|---|---|---|---|---|---|---|---|---|
| 12 | 37 | F | 1.40* | 0.75 | neg | isopropanol (0.24 g/100 mL) acetone (0.13g/100 mL) | subject found dead in bed; Hx of depression & alcohol abuse | isopropyl alcohol ingestion | undetermined | unknown |
| 13 | not known | F | 1.73* | 0.93 | neg | -- | subject called 911, having trouble breathing in motel room; found DOA; Rx's in subject's purse include propoxyphene/APAP, lorazepam, dicyclomine, fluoxetine, dilacor, HCTZ/tramterene | cardiac arrhythmia due to dilated cardiomyopathy ; contributory causes: intramuscular bridging of coronary artery, fatty liver, acute fluoxetine poisoning | accident | peripheral |
| 14 | 42 | M | 3.67* | 0.38 | 0.03 | nicotine/cotinine | long Hx of depression; found deceased by apartment manager | pharmaceutical overdose | suicide | unknown |
| Spleen | | | | | | | | | | |
| 15 | 41 | M | 6.66* | 20.27 | 0.05 | trazodone (2.65) | friends had not seen decedent recently & became alarmed at foul smell pervading hallway outside his apartment; found dead; body in advanced decomposition | arteriosclerotic cardiovascular disease | arteriosclerotic cardiovascular disease | N/A |

* Concentration above therapeutic range.

### 3.4.3. Fluvoxamine.

After approximately one week of multiple oral fluvoxamine dosing of 100-300 mg/day in 30 normal volunteers yielded steady-state plasma levels ranging from 0.09-0.55 mg/L (78). In elderly patients, aged 66-73 years, mean plasma fluvoxamine concentrations were 40% higher than in younger subjects, aged 19-35 years. Concentrations of its active metabolite, fluvoxamine acid, were not reported in either of these studies. As with the other SSRI's previously discussed, fluvoxamine acid is selective for serotonin reuptake inhibition.

Like fluoxetine, fluvoxamine exhibits autoinhibition, and may be involved in metabolic interactions involving CYP1A2, 2D6, and/or 3A4 when other substrates for these isozymes are present, as fluvoxamine is a potent inhibitor of all three. There have been many reports of bleeding disorders involving fluvoxamine (14). In addition, fluvoxamine may be involved in cases of serotonin syndrome, although no such reports have yet been published. The authors of a comparative study of the incidence of hyponatremia in patients taking SSRI's found that 11 out of 736 cases of hyponatremia and SIADH involved fluvoxamine, compared to 554 involving fluoxetine (121). There have been 354 cases of deliberate or accidental overdose involving fluvoxamine in clinical trials, 19 of which had fatal outcomes (78). Two of these deaths involved patients taking other medications. Fluvoxamine was not detected in any of the case samples analyzed in this study.

### 3.4.4. Sertraline.

Serum sertraline levels of 0.03-0.19 mg/L were measured in patients after 14-day dosing at oral doses of 50-200 mg (122). Patients on chronic oral daily doses of 100-300 mg achieved steady-state plasma levels ranging from 0.02-0.21 mg/L of sertraline, with *n*-desmethylsertraline (active metabolite) concentrations averaging 167% of the parent drug concentration (123). *N*-desmethylsertraline, like norfluoxetine, is selective for serotonin reuptake inhibition, although it possesses only 10-20% of the pharmacological activity of the parent drug (22).

Like fluoxetine, side effects commonly associated with sertraline are bleeding disorders, SS, and combined drug overdose, likely a consequence of isoenzyme inhibition, as sertraline is a substrate for CYP3A4 and inhibits both 3A4 and 2D6. The study of incidence of hyponatremia and SIADH (121) showed that 86 out of 736 cases involved sertraline. Cases of SS involving sertraline, although less common than those involving fluoxetine, have been reported in which a variety of other drugs, including phenelzine, isocarboxazid, and tranylcypromine were present (124-127).

No sertraline-only overdoses have previously been reported in the literature. In a case analyzed by this laboratory, an adult male who committed suicide by drug overdose was found to have sertraline and *n*-desmethylsertraline blood levels of 0.61 and 1.60 mg/L, respectively (diphenhydramine was also present at 0.58 mg/L) (34). In another suicidal overdose, a sertraline level of 1.56

mg/L was measured in the blood of a 51 year-old woman (92). The *n*-desmethylsertraline concentration was not determined however, due to the authors' inability to obtain a standard. Bromazepam (382 mg/L) and levomepromazine (423 g/L) were also present in that case. A report of the distribution of sertraline in seven postmortem cases not caused by sertraline intoxication measured central blood concentrations ranging from 0.23-0.46 mg/L of sertraline and 0.08-0.99 mg/L of *n*-desmethylsertraline, and noted high liver concentrations of both compounds relative to those in other tissues (93).

There were more sertraline-related fatalities (Table III.8) over the course of this study than any other SSRI. Cases 1, 7, 10, 11, 15, and 18-20 are all multiple drug intoxications where sertraline likely played a direct role in fatality. Increased blood sertraline levels caused by competitive inhibition of CYP2D6 leading to toxic side effects may have been a contributing factor in Cases 7, 10, and 18-20, as propoxyphene, codeine, hydrocodone, dextromethorphan, and oxycodone are all substrates for 2D6 (in addition to amitryptiline and nortryptiline), and diltiazem inhibits 2D6. It is also possible that inhibition of 3A4 in Cases 1, 15, and 18-20 played a role due to the presence of bupropion's morpholinol metabolite in Case 1, and of trazodone in Cases 15 and 18-20. Alcohol was also present at high concentrations in Cases 18 and 20, and may have contributed to a certain degree to these deaths.

## Table III.8. Case Information on Decedents Testing Positive for Sertraline.

| # | Age | Sex | Sert (mg/L) | *n*-dSert (mg/L) | Alcohol (g/100 mL) | Other Drug Use (mg/L) | Circumstances Surrounding Death | Cause of Death | Manner of Death | Central or Peripheral Blood? |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 35 | F | -- | 1.09 | neg | bupropion (7.86) threoamino alcohol (6.77) bupropion morpholinol metabolite (0.93) | Hx of depression & suicide attempts; went into cardiac arrest in ER after taking pills, unable to be resuscitated; incidental findings: coronary atherosclerosis, cardiomegaly, thyroid follicular adenoma | cardiorespiratory arrest 2° to drug overdose | not given | unknown |
| 2 | 20 | M | 0.09 | 0.11 | neg | -- | Hx of mental illness & substance abuse; subject found hanging at home; Rx medications incl Haldol, Zoloft | hanging | suicide | unknown |
| 3 | 45 | F | 0.13 | 0.09 | 0.20 | morphine (0.44) | Hx of alchol abuse, suspected of having helped herself to cancer patient's liquid pain medication | acute intoxication by combined effects of opiates & ethanol | accident | peripheral |
| 4 | 50 | M | 0.13 | 0.22 | neg | thioridazine (0.25) mesoridazine (0.17) | Hx of alcohol abuse & possible seizure; sudden, unexpected death, autopsy finding: acute subdural hematoma | acute subdural hematoma secondary to blunt head trauma consistent with a fall | accident | unknown |
| 5 | 55 | M | 0.16 | 0.13 | neg | morphine (0.13) benzoylecgonine (0.59) methadone (0.77) meprobamate (21.8) | subject w/ Hx of drug abuse found hanging in motel room; had recently discussed suicide | hanging | suicide | peripheral |

Table III.8 (Continued).

| # | Age | Sex | Sert (mg/L) | *n*-dSert (mg/L) | Alcohol (g/100 mL) | Other Drug Use (mg/L) | Circumstances Surrounding Death | Cause of Death | Manner of Death | Central or Peripheral Blood? |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 39 | F | 0.21* | – | 0.10 | nortriptyline (1.06) hydrocodone (0.28) temazepam (1.30) acetominophen (215) | found dead w/ plastic bag over head | asphyxia due to suffocation by plastic bag | suicide | peripheral |
| 7 | 46 | F | 0.21* | 0.24 | neg | amitriptyline (0.84) nortriptyline (0.47) | Hx of marital discord, separated from husband; prior to death found letter from husband to other woman, on sertraline & amitriptyline Rx | acute combination prescription drug intoxication | suicide | unknown |
| 8 | 46 | F | 0.22* | 1.25 | neg | lidocaine carbamazepine (3.10) | Hx of insulin-dependent diabetes, heart trouble | probably atherosclerotic cardiovascular disease, w/ contributory condition of diabetes mellitus | natural | unknown |
| 9 | 54 | F | 0.24* | – | neg | – | jumped in front of semi-truck | multiple visceral lacerations & skeletal fractures due to blunt impact to head, trunk, & extremities | suicide | peripheral |
| 10 | 37 | M | 0.26* | 0.08 | neg | oxycodone (1.00) | Hx of hepatitis C, multiple drug overdose, depression over recent separation from wife; subject took several vicodin from friend's prescription bottle; found dead at home | possible acute intoxication due to combined effects of oxycodone & sertraline | probable accident | unknown |

Table III.8 (Continued).

| # | Age | Sex | Sert (mg/L) | *n*-dSert (mg/L) | Alcohol (g/100 mL) | Other Drug Use (mg/L) | Circumstances Surrounding Death | Cause of Death | Manner of Death | Central or Peripheral Blood? |
|---|---|---|---|---|---|---|---|---|---|---|
| 11 | 47 | F | 0.29* | 1.46 | neg | morphine (0.51) diphenhydramine (0.25) methadone (0.15) | previous Hx of Rx drug overdose; found deceased at home in bathroom; Rx incl. alprazolam, zoloft, cyclobenzaprine, maxide, naproxen | acute multiple drug intoxication | undetermined | unknown |
| 12 | 46 | M | 0.30* | -- | 0.09 | methadone (0.27) doxapin (0.10) diazepam (0.05) nordiazepam (0.28) morphine (<0.01) | found dead in bedroom at home, on sick leave due to back injury; autopsy findings incl. coronary atherosclerosis; drug levels found not indicative of overdose | atherosclerotic cardiovascular disease w/ focal moderate to severe coronary atherosclerosis | natural | peripheral |
| 13 | 87 | F | 0.50* | 0.50 | neg | diazepam (<0.05) | subject died in hospital | chronic obstructive pulmonary disease w/ focal pneumonia & cardiomegaly w/ congestive heart failure | natural | peripheral |
| 14 | 75 | M | 0.57* | 0.55 | 0.12 | amphetamine (2.10) fluoxetine (0.67) norfluoxetine (0.29 | found diseased at home; body moderatelyt decomposed when found | coronary artery atherosclerosis | natural | unknown |
| 15 | 38 | M | 0.75* | 0.38 | neg | diphenhydramine (1.20) loxapine (1.40) trazodone (0.50) | stricken in county jail, wrote suicide note to wife & took apparent drug overdose; resuscitation attempts unsuccessful | multiple drug intoxication | suicide | peripheral |

Table III.8 (Continued).

| # | Age | Sex | Sert (mg/L) | *n*-dSert (mg/L) | Alcohol (g/100 mL) | Other Drug Use (mg/L) | Circumstances Surrounding Death | Cause of Death | Manner of Death | Central or Peripheral Blood? |
|---|---|---|---|---|---|---|---|---|---|---|
| 16 | 49 | M | 0.75* | 0.82 | neg | verapamil (0.07) norverapamil (0.16) | subject found in his motor vehicle with gunshot wound to head; was involved in shooting 2 people earlier | penetrating gunshot wound to head | suicide | unknown |
| 17 | 51 | M | 1.25* | 1.16 | neg | cocaine (1.4) benzoylecgonine (3.7) nordiazepam (<0.1) | subject fell in trailer, hitting head against ledge | acute cardio-pulmonary arrest due to fractured 3rd cervical vertebra | accident | unknown |
| 18 | 39 | F | 1.76* | 0.15 | 0.19 | propoxyphene (6.60) norpropoxyphene (0.87) codeine (7.00) acetominophen (521.00) ibuprofen (37.00) hydrocodone (1.28) diphenhydramine (1.09) trazodone (1.41) | subject found dead in motor vehicle; identified as official missing personas of 2 days prior | acute intoxication due to combined effects of acetominophen, codeine, propoxyphene, hydrocodone, trazodone, sertraline, ibuprofen, diphenhydra-mine, & ethanol | probable suicide | unknown |
| 19 | 45 | F | 1.76* | 0.57 | neg | diltiazem (3.35) temazepam (7.75) trazodone (24.32) | depression Hx w/ apparent multiple drug overdose | acute intoxication by combined effects of trazodone & benzodiazepines | suicide | peripheral |

Table III.8 (Continued).

| # | Age | Sex | Sert (mg/L) | *n*-dSert (mg/L) | Alcohol (g/100 mL) | Other Drug Use (mg/L) | Circumstances Surrounding Death | Cause of Death | Manner of Death | Central or Peripheral Blood? |
|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 44 | F | 2.52* | 4.05 | 0.18 | dextromethorphan (0.49) promethazine (0.98) antipyrine cocaine (<0.05) cocaethylene (<0.05) benzoylecgonine (0.57) trazodone (0.35) | Hx of chronic alcoholism; autopsy findings incl pill fragments in stomach | acute combined drug intoxication; contributory cause: advanced micronodular cirrhosis w/ prominent splenomegaly | accident | peripheral |

* Concentration above therapeutic range.

*n*-desmethylsertraline were studied in Case 20, and levels detected are reported in Table III.4. Of note in this case is the significantly higher concentration of both compounds in the liver compared to other tissues. If competitive inhibition was a factor, the high concentration of sertraline is most likely a result of this inhibition due to the presence of other substrates of 2D6 and/or 3A4 (dextromethorphan, trazodone) in the subject's blood. The combined serotonergic activity of multiple drugs in Cases 7, 18, and 20 is also likely to have been important.

Death in Cases 1, 8, and 12-14 might have been precipitated by the cardiovascular effects of serotonin due to the presence of serotonergic drugs in their blood. The contracting action of excess circulating serotonin causing vasoconstriction may have led to cardiorespiratory arrest due to these subjects' histories of cardiovascular disease.

### 3.4.5. Paroxetine.

In 29 healthy subjects given a single 20 mg oral dose of paroxetine, peak plasma levels of 0.01-0.03 mg/L were measured within 3-8 hours (128). Steady-state plasma levels averaging 0.06 mg/L were measured in 15 healthy adult males who received a single 30 mg/day oral dose for 30 days (78). Paroxetine is extensively biotransformed to largely inactive metabolites via oxidation, methylation, and conjugation.

One case of SS involving paroxetine has been reported in the literature (25). In this case the subject, a 51-year-old man,

developed symptoms of SS which later dissipated after self-medicating with Nyquil (containing dextromethorphan) while also taking paroxetine. This subject also had significant peripheral vascular disease, which may have played a role in his death. Patients such as this one with peripheral vascular or cardiovascular disease are particularly vulnerable to toxic side effects of paroxetine and other SSRI's due to vasoconstriction potentiated by excess platelet 5-HT (11).

Like fluoxetine, norfluoxetine, and fluvoxamine, paroxetine exhibits autoinhibition which may potentiate therapeutic or toxic effects of the drug (14). Hyponatremia and SIADH are common side effects associated with paroxetine. The authors of the study of incidence of hyponatremia and SIADH mentioned earlier (121) found that 91 out of 736 cases involved paroxetine, compared with 554 involving fluoxetine, 86 involving sertraline, and 11 involving fluvoxamine. Isoenzyme metabolism may also play a role in toxicity in cases involving other drugs which are CYP2D6 or 3A4 substrates. Paroxetine is a substrate and potent inhibitor of 2D6 and a weak inhibitor of 3A4.

No fatal overdoses involving paroxetine alone have been reported in the literature. In a case of assisted suicide analyzed in this laboratory, a 72-year-old woman on paroxetine ingested 100 phenobarbital capsules, 50 amitriptyline, and 1 dramamine tablet with a glass of champagne. Paroxetine levels in the subject's central and peripheral blood, liver, bile, and gastric contents were

5.6, 1.4, 37.0, 2.6, and 1.8 mg/L (94). Paroxetine could not be detected in vitreous fluid in that case. In another fatality, a woman who intentionally overdosed on paroxetine was found to have blood and liver levels of 0.24 mg/L and 3.5 mg/kg, respectively. Amitriptyline was also present, at concentrations of 0.43 mg/L and 6.80 mg/kg (95).

Both paroxetine-related deaths in this study (Table III.9) were certified as cardiac-related deaths, although the paroxetine concentration in both cases was above therapeutic levels. The contracting action of serotonin due to the presence of several serotonergic drugs likely played a role in both cases, possibly heightened by increased blood paroxetine levels caused by competitive inhibition of CYP2D6. A study of the postmortem tissue distribution of Case 1 revealed the highest paroxetine concentrations in the urine, bile, and central blood, respectively. As with the trazodone cases, the central blood concentration was higher than that for peripheral blood.

### 3.4.6. Venlafaxine.

Following a single oral 150 mg dose of venlafaxine, peak serum concentrations ranging 0.08-0.29 mg/L were measured within approximately 2 hours post-dose. Multiple-dose trials in which patients were given 75 mg every 8 hours yielded plasma levels of between 0.07-0.27 mg/L of venlafaxine and 0.24-0.52 mg/L of *o*-desmethylvenlafaxine, the active metabolite (129). Predicted steady-state plasma concentrations of both compounds in normal

## Table III.9. Case Information on Decedents Testing Positive for Paroxetine.

| # | Age | Sex | Par (mg/L) | Other Drug Use (mg/L) | Alcohol (g/100 mL) | Circumstances Surrounding Death | Cause of Death | Manner of Death | Central or Peripheral Blood? |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 39 | M | 0.20* | methamphetamine (0.85) amphetamine (0.10) nortriptyline (0.67) carbamazepine (6.20) nortriptyline met | neg | found dead at residence; Hx of amphetamine & cocaine abuse | arteriosclerotic cardiovascular disease | natural | peripheral |
| 2 | 51 | F | 3.84* | dextromethorphan (0.21) hydroxyzine (4.4) trazodone (0.78) | neg | Hx of depression | probable myocardial infarction due to arteriosclerotic cardiovascular disease | undetermined | unknown |

* Concentration above therapeutic range.

subjects receiving 150 mg daily are 0.07 and 0.25 mg/L, respectively (130). Both venlafaxine and *o*-desmethylvenlafaxine potently inhibit neuronal serotonin and norepinephrine reuptake, although serotonin reuptake is inhibited less by venlafaxine than by other SSRI's (4). The most common toxic effects associated with venlafaxine are sustained hypertension, orthostatic hypotension, cardiac arrhythmia, seizures, and coma (22). Venlafaxine has also been implicated in serotonin syndrome in the literature, in combination with MAOI's (131). As with the other drugs, isoenzyme metabolism may figure significantly in cases of toxicity associated with venlafaxine when other drugs which are substrates for or inhibitors of CYP2D6 are present, as venlafaxine has been shown to be metabolized to *o*-desmethylvenlafaxine by this isozyme.

Several venlafaxine-related fatalities have been reported in the literature. In central blood from three cases of suicide caused by acute drug intoxication, venlafaxine and *o*-desmethylvenlafaxine concentrations ranging from 6.6-84.0 and 15.0-50.0 mg/L were measured (96). Tissue distribution was studied in two of the cases, both of which had other drugs present. Parent drug and metabolite levels measured in peripheral blood, bile, urine, liver, and kidney samples were 46 and 7.1 mg/L in peripheral blood, 100-290 and 32-52 mg/L in bile, 150-640 and 59-310 mg/L in urine, 34-430 and 54-140 mg/kg in liver, and 210 and 43 mg/kg in kidney. In another study of tissue distribution of venlafaxine,

peripheral and central blood, vitreous fluid, liver, and urine samples were analyzed. Levels of venlafaxine and *o*-desmethylvenlafaxine were 17.0-65.0 and 7.1.0 mg/L in peripheral blood, 30.0-85.0 and 5.6 mg/L in central blood, 11.0-23.0 and 0.68 mg/L in vitreous fluid, 220.0-425.0 and 11.0 mg/kg in liver, and 20.0-73.0 and 28.0 mg/L in urine (97). In one other study of postmortem venlafaxine tissue distribution (99), the authors analyzed heart blood, liver, bile, and gastric contents, and obtained similar results.

Only one of the venlafaxine-related deaths (Table III.10, Case 4) can be attributed to acute combination drug intoxication. Competitive inhibition of 2D6 by nortriptyline may be partially responsible for the elevated venlafaxine and low *o*-desmethylvenlafaxine concentrations in this case. As with earlier cases, the combined serotonergic activity of both drugs present is important to keep in mind. Postmortem tissue distribution of venlafaxine and *o*-desmethylvenlafaxine was studied in Cases 2 and 3. Relative concentrations among the various tissues differ between the two cases, so it is difficult to draw many conclusions. However, in both cases, the liver concentration was higher than that of either central or peripheral blood.

### 3.4.7. Effect of SSRI's on driving.

The presence of alcohol and/or other drugs seems to be almost universal in cases involving SSRI's. For example, four drug-impaired drivers, all of whom had therapeutic concentrations of

## Table III.10. Case Information on Decedents Testing Positive for Venlafaxine.

| # | Age | Sex | Ven (mg/L) | ODV (mg/L) | Alcohol (g/100 mL) | Other Drug Use (mg/L) | Circumstances Surrounding Death | Cause of Death | Manner of Death | Central or Peripheral Blood? |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 38 | M | 0.227* | 0.161 | 0.15 | diazepam ((<0.10) nordiazepam (0.12) | subject shot in confrontation w/ police officer | perforating gunshot wound to chest that injured both lungs, aorta, & liver | homicide | unknown |
| 2 | 47 | F | 0.75* | 0.28 | neg | – | driver in traffic accident; subject went out of control & struck concrete barrier, then hit by truck | basilar skull fractures w/ intraventricular hemorrhage w/ multiple rib fractures & lacerations of lungs due to blunt impact to head & trunk | accident (traffic) | peripheral |
| 3 | 50 | F | 0.97* | 1.03 | neg | – | Hx of previous suicide attempts & treatment for mental illness; subject found lying on floor of residence w/ bag over head | asphyxia due to suffocation by plastic bag | suicide | peripheral |
| 4 | 38 | M | 13.93* | 0.25 | 0.22 | nortriptyline (1.60) | depression Hx w/ suicide attempts, found dead at residence; 4 empty medication bottles found on nearby table (Effexor, nortriptyline, & temazepam) | acute intoxication due to the combined effects of venlafaxine, ethanol, & nortriptyline | suicide | unknown |

* Concentration above therapeutic range.

different SSRI's in their blood also had significant concentrations of alcohol and/or other drugs present (Table III.11). Numerous studies of the impact of fluoxetine in combination with benzodiazepines, amitriptyline, and/or alcohol on psychomotor performance have been performed (132-140). Methods varied across these studies, with both single- and multiple-dose regimens being used. While the findings in these studies varied slightly, the authors of all studies concluded that concomitant administration of either multiple or single doses of fluoxetine with acute ethanol did not impair psychomotor performance. Indeed, some parameters were shown to improve with fluoxetine and acute alcohol taken together compared to acute alcohol alone (133), and fluoxetine alone did not appear to affect psychomotor performance. However, both single- and multiple-doses of fluoxetine with benzodiazepines did appear to impair psychomotor test performance more than benzodiazepines alone (139, 140).

All of these studies used lower doses of fluoxetine, consistent with what one would expect in cases of therapeutic use of the drug. Therefore, although the contribution of SSRI's to driving impairment when alcohol is present appears to be minor based on the findings in these studies, impairment may or may not occur at elevated concentrations. This is a matter which has not yet been investigated in the literature.

Table III.11. Case Information on Drug-impaired Drivers Testing Positive for Serotonergic Drugs.

| # | Age | Sex | Sert (mg/L) | *n*-dSert (mg/L) | Fluox (mg/L) | Norflu (mg/L) | Ven (mg/L) | ODV (mg/L) | Alcohol (g/100 mL) | Other Drugs Present (mg/L) | Circumstances Surrounding Case |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 60 | F | 0.50 | 0.59 | – | – | – | – | neg | nortriptyline (1.00) | Hx of depression; Rx incl digoxin, amitriptyline, Ativan |
| 2 | 27 | M | – | – | 0.30 | 0.13 | – | – | 0.01 | zolpidem (<0.1) | subject rear-ended stopped vehicle; smelled of alcohol & stated he took some sleeping pills (Ambien) |
| 3 | 34 | F | – | – | – | – | 0.06 | 0.18 | 0.05 | alprazolam (0.36) | driver in 1-car accident; admitted ingesting 40 Xanax tablets & other drugs, & had been consuming alcohol |
| 4 | 36 | F | – | – | – | – | 0.13 | 0.83 | neg | phenobarbital (20.60) ibuprofen (15.90) | driver in 2-car accident |

### 3.4.8. Categories of deaths related to atypical antidepressants.

The drug-related deaths in this study can be neatly divided into three groups. Suicides, accidents, or homicides in which the cause of death is not directly related to drug use comprise the first group. In these cases, concentrations of serotonergic drugs are at or below therapeutic levels. The second group includes all combined drug intoxications or overdoses, regardless of the particular drug. Concentrations of drugs present in these cases are at or above therapeutic concentrations, and more than one drug is present in each case. Because of this, isoenzyme metabolism and serotonergic activity of all drugs present needs to be taken into consideration. The last group is made up of subjects with cardiovascular disease. Serotonergic drug levels are below, at, or above therapeutic levels, More than one drug is present in all but one case from this group, so serotonergic activity and isoenzyme metabolism are both important to keep in mind when interpreting drug levels.

## 3.5. Conclusions.

Selective Serotonin Reuptake Inhibitors are a new class of antidepressants preferentially prescribed for a variety of conditions due to the low incidence of untoward side effects associated with their use. Trazodone and bupropion, while not categorized as SSRI's, are also new drugs which possess atypical modes of action compared to the classical tricyclic antidepressants,

and which, like the SSRI's are considered relatively safe due to the specificity of their effects. The active metabolites of the SSRI's and trazodone are selective for inhibition of serotonin reuptake like their parent drugs, and those of bupropion inhibit dopamine and norepinephrine reuptake. Many of these drugs have large volumes of distribution like the tricyclics, but also have much longer half-lives than the earlier antidepressants.

The recent increase in popularity of serotonergic drugs has necessarily brought with it an increase in fatalities where such drugs play a role. SS is a potentially fatal disorder which is infrequently recognized, and it is typically the result of administration of a combination of drugs which affect serotonin levels. In fact, it is the most common life-threatening consequence of SSRI overdose. Several such fatalities have been discussed in this chapter. Concentrations of atypical antidepressants in cases of SS are most likely to be at or above the therapeutic range (>0.49 mg/L of trazodone, >0.01 mg/L of bupropion, >0.06 mg/L of fluoxetine, >0.02 mg/L of fluvoxamine, >0.03 mg/L of sertraline, >0.03 mg/L of paroxetine, and >0.07 mg/L of venlafaxine). In cases where more than one drug with serotonin reuptake inhibition ability is present, all drug levels may be within the therapeutic range. It is recommended that clinicians maintain heightened awareness to help minimize the prescription of medication combinations which are likely to induce SS.

Many of the atypical antidepressants and/or their metabolites

are substrates or inhibitors of CYP2D6, 3A4, or 1A2 and are usually found in combination with other drugs which are substrates for one of these isoenzymes. As with tramadol, therefore, it is important to consider isoenzyme metabolism when interpreting blood levels of these drugs. In fatalities involving metabolic interactions, drug levels are again likely to be at or above the therapeutic range, but metabolite concentrations may be above those of the parent drug in cases of metabolic induction or below those of the parent drug in cases of metabolic inhibition. A simple method for determining whether an SSRI ingestion may have been an acute overdose has elsewhere been suggested for fluoxetine (14). If the fluoxetine/norfluoxetine ratio is >2:1, chances are that it was an acute ingestion.

The potentially lethal combination of vascular disease and SSRI's resulting in severe vasoconstriction has earlier been discussed, and there is evidence among one-fifth of the cases here reported that this combination contributed to the deaths. In these cases, drug levels may be at or below the therapeutic range (<1.60 mg/L of trazodone, <0.45 mg/L of fluoxetine, <0.42 mg/L of fluvoxamine, <0.19 mg/L of sertraline, <0.06 mg/L of paroxetine, and <0.25 mg/L of venlafaxine). Bupropion use has not been linked to hypertension or orthostatic hypotension like the other atypical antidepressants, so this mechanism of toxicity is not likely to occur in bupropion-related fatalities.

Psychiatric care givers should watch for suicidal ideation in

patients taking multiple serotonergic drugs concomitantly, as several of the cases discussed in this report were suicides caused by drug overdose. It is likely that the number of such fatalities will increase in the future. The findings in this report should provide a tool for medical examiners to recognize the various mechanisms of toxicity associated with atypical antidepressants from scene investigation findings and toxicology reports. It is also anticipated that this study will highlight the increasing occurrence of and circumstances surrounding adverse drug reactions associated with such drugs.

# CHAPTER 4: EVALUATION OF A METHOD FOR DETERMINING SEROTONIN AND METABOLITES IN POSTMORTEM SERUM

## 4.1. Introduction.

A significant number of cases discussed in earlier chapters involved subjects with cardiovascular disease or some other chronic heart condition. Indeed, one-fifth of the subjects in cases involving atypical antidepressants fit this profile. It is very likely that the action of platelet 5-HT may play an important role in fatalities involving such subjects by causing vasoconstriction. As discussed in Chapter 1, 5-HT induces a variety of responses by the heart which result from activation of various 5-HT receptor subtypes. 5-HT has chronotropic and inotropic effects, and it increases the vasoconstriction produced by noradrenaline, angiotensin II, and histamine, which reinforce the hemostatic response to 5-HT (3, 7). The incidence of this effect in decedents is difficult to ascertain, as such deaths are usually certified as natural deaths due to the decedent's heart condition. However, the ability to measure 5-HT in postmortem blood immediately after death might help to elucidate the magnitude of this effect. This would also be useful in subjects who develop serotonin syndrome, as blood levels in such cases are often within the therapeutic range, so pathologists might not appreciate the role of such drugs in a fatality.

Analytical methods for 5-HT in brain tissue, cerebrospinal fluid, urine, whole blood, and plasma have all been published, and were

discussed in Chapter 1 (40-48). Most of these employ HPLC with either fluorescence or electrochemical detection, because they require little sample handling and provide for fast analysis. GC/MS methods have also been developed for identification of 5-HT in cerebrospinal fluid and brain tissue, (49-53), but the GC/MS methods are generally not sensitive enough for determination of 5-HT and metabolites in platelet-poor or platelet-free plasma. While brain 5-HT concentrations are on the order of several hundred nanomoles/L, plasma 5-HT levels are usually in the range from 5-15 nanomoles/L. The authors of one study (141) hypothesized that the range of 5-HT concentrations previously reported in the literature for platelet-poor plasma (PPP) (1.4-28 ng/ml) are actually on the high side. These authors found that there appeared to be a significant release of platelet 5-HT at the time the blood was drawn if anticoagulant was not added directly to the syringe (PPP levels 2.8-fold higher in such samples). They also observed a small but discernible release of platelet 5-HT attributable to the centrifugation process when extremely high speeds (12,000 x g) were used. Hourani and Cusack noted in a more recent study that stirring at speeds of 1,000 rpm (280 x g), were sufficient to cause platelets to aggregate, a process promoted by 5-HT release (4). However, in the context of the findings of Anderson and colleagues, release of 5-HT during centrifugation appears to only become significant at higher speeds.

The application of an analytical method for detection of plasma 5-HT is much more complex in postmortem specimens. Hemolysis (destruction of red blood cells and resultant escape of hemoglobin) occurs at death, making it difficult to get a platelet-poor sample, as hemolysis negates the possibility of obtaining a clear supernate and therefore obscures the separation of plasma and platelets obtained from centrifugation. No investigation of postmortem platelet rupture has been reported in the literature, so it is unclear if this is an important factor of postmortem serum 5-HT concentrations. In addition to postmortem hemolysis, repeated thawing and freezing of samples sent to the toxicology laboratory for multiple analyses tends to rupture any still intact red blood cells and may also cause degradation of 5-HT prior to sample preparation for assay. Postmortem stability studies of 5-HT in brain have shown that it is unstable at room temperature once removed from intact tissue (35-41).

In this chapter, the feasibility of using an analytical method (48) using high performance liquid chromatography (HPLC) with electrochemical detection for determination of 5-HT, 5-hydroxyindole-3-acetic acid (5HIAA) and indole-3-acetic acid (IAA) in postmortem plasma is discussed. The method was applied to a group of clear serotonergic drug overdose cases, and the results compared to those from a group of case samples where no drug was present. The stability of 5-HT and its metabolites in PPP was also investigated.

## 4.2. Materials and Methods.

IAA, 5-HT, 5HIAA, bufotenine (internal standard), and ascorbic acid were obtained from Sigma. All other reagents were analytical grade or better, and were obtained from J.T. Baker or Fisher.

High Performance Liquid Chromatography (HPLC) was carried out using a reversed-phase isocratic method with electrochemical detection developed by Martínez, Artigas, Suñol, Tusell, and Gelpí and discussed elsewhere (48). The system consisted of a Gilson 305 pump and 805 manometric module (Gilson, Middleton, WI), a Rheodyne sample injector (Model 7161, Rheodyne) equipped with a 20-uL loop, a dual parallel LC-4B amperometric detector fitted with a TL-5 glassy carbon electrode assembly (Bioanalytical Systems, West Lafayette, IN), a Newguard RP-8 guard column (7 micron particle size, 20 X 3.2 mm I.D., Rainin, Woburn, MA), and a μBondapak $C_{18}$column (300 X 3.9 mm I.D., Waters Assoc., Milford, MA). The mobile phase consisted of a 94/6% (by volume) mixture (pH 4.8) of citric acid (30 mmol/L), disodium hydrogen phosphate (60 mmol/L) and methanol, at a flow rate of 1.5 mL/min. The electrochemical detector was operated at a potential of +0.55 V vs the Ag/AgCl reference electrode. This potential was chosen after investigation of the electrochemical response of a fixed amount of serotonin to applied electrode potentials ranging from 0.2 V to 0.8 V (Figure IV.1).

Because most circulating serotonin is contained in blood platelets (141), an attempt at preparing platelet-poor samples was

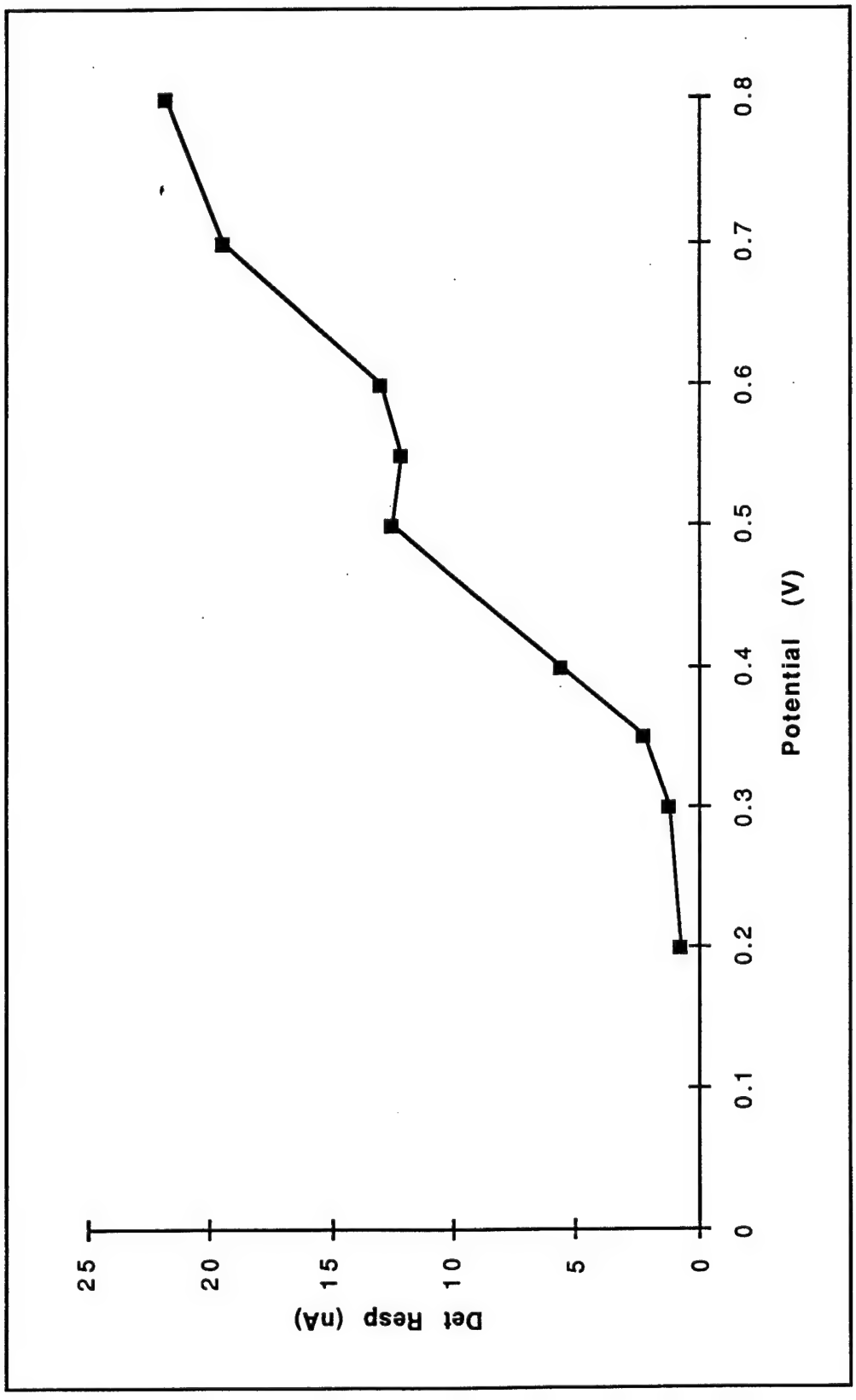



Figure IV.1. Hydrodynamic voltammogram for serotonin.

made according to the following method. Blood samples collected at autopsy during the investigation of twenty unrelated fatalities were each placed in separate 10-mL vials containing sodium fluoride and potassium oxalate (Vacutainer; Becton Dickinson, New Jersey) and immediately frozen (4°C). The samples were allowed to thaw to room temperature, and 1 mL aliquots of each sample were centrifuged (2000 rpm, 25 °C, 10 min). This speed corresponds to 1118 x g according to the equation (147)

$$g = (11.18)r(n/1000)^2$$

where g = relative centrifugal force

r = spinning radius in cm

n = revolutions per minute

Published methods discussing preparation of PPP (47-49) call for centrifugation at 1000 x g to avoid release of platelet 5-HT as described earlier. The supernates were carefully removed and stored at 4°C until analysis was performed.

Protein precipitation was performed using a procedure based on that described by Artigas and co-workers (47, 48). Platelet-poor specimens (200 uL) were spiked with 100 uL of 200 ng/mL bufotenine, the internal standard. Protein precipitation was achieved by adding 50 uL of 1 g/L ascorbic acid and 300 uL of 50 g/L trichloroacetic acid to the mixture. After vortex-mixing the samples and allowing them to stand at 4°C for 5 min, they were centrifuged at 2000 rpm for 15 min, and 20 uL of the supernate was directly injected onto the HPLC. Since it was not possible to

centrifugate samples at 4°C, trichloroacetic acid and ascorbic acid were refrigerated prior to use to keep the samples cold.

**4.3. Results and Discussion.**

A chromatogram showing the separation of 5-hydroxyindole-3-acetic acid, 5-HT, IAA, and bufotenine is shown in Figure IV.2. The mean concentrations of 5-HT, 5HIAA, and IAA in cases where no drug was present was 0.06 ±0.08, 0.11±0.11, and 0.01±0.01 umol/L, respectively. In SSRI overdose cases the average concentrations were 49.24±92.20, 0.01±0.01, and 0.15±0.26 umol/L. Table IV.1 lists the results of analysis of the samples in which no drug was present. The results of analysis of the SSRI overdose cases, as well as concentrations of drugs present and circumstances surrounding each death are listed in Table IV.2.

Because postmortem stability of 5-HT in blood samples stored for several months was expected to affect results of this assay, blood

Table IV.1. Serotonin (5-HT) Levels in Subjects with no Drug Present in Their Blood*.

| Sample # | Sex | Amount 5-HT | Amount 5HIAA | Amount IAA | Drugs present |
|---|---|---|---|---|---|
| 1 | M | 0.02 | 0.00 | 0.00 | none |
| 2 | M | 0.000 | 0.00 | 0.00 | none |
| 3 | M | 0.00 | 0.00 | 0.00 | none |
| 4 | M | 0.03 | 0.00 | 0.00 | none |
| 5 | M | 0.02 | 0.00 | 0.00 | none |
| 6 | M | 0.06 | 0.28 | 0.00 | none |
| 7 | M | 0.28 | 0.18 | 0.00 | none |
| 8 | M | 0.03 | 0.25 | 0.02 | none |
| 9 | F | 0.06 | 0.09 | 0.00 | none |
| 10 | M | 0.06 | 0.07 | 0.00 | none |

* (Concentrations in umol/L).

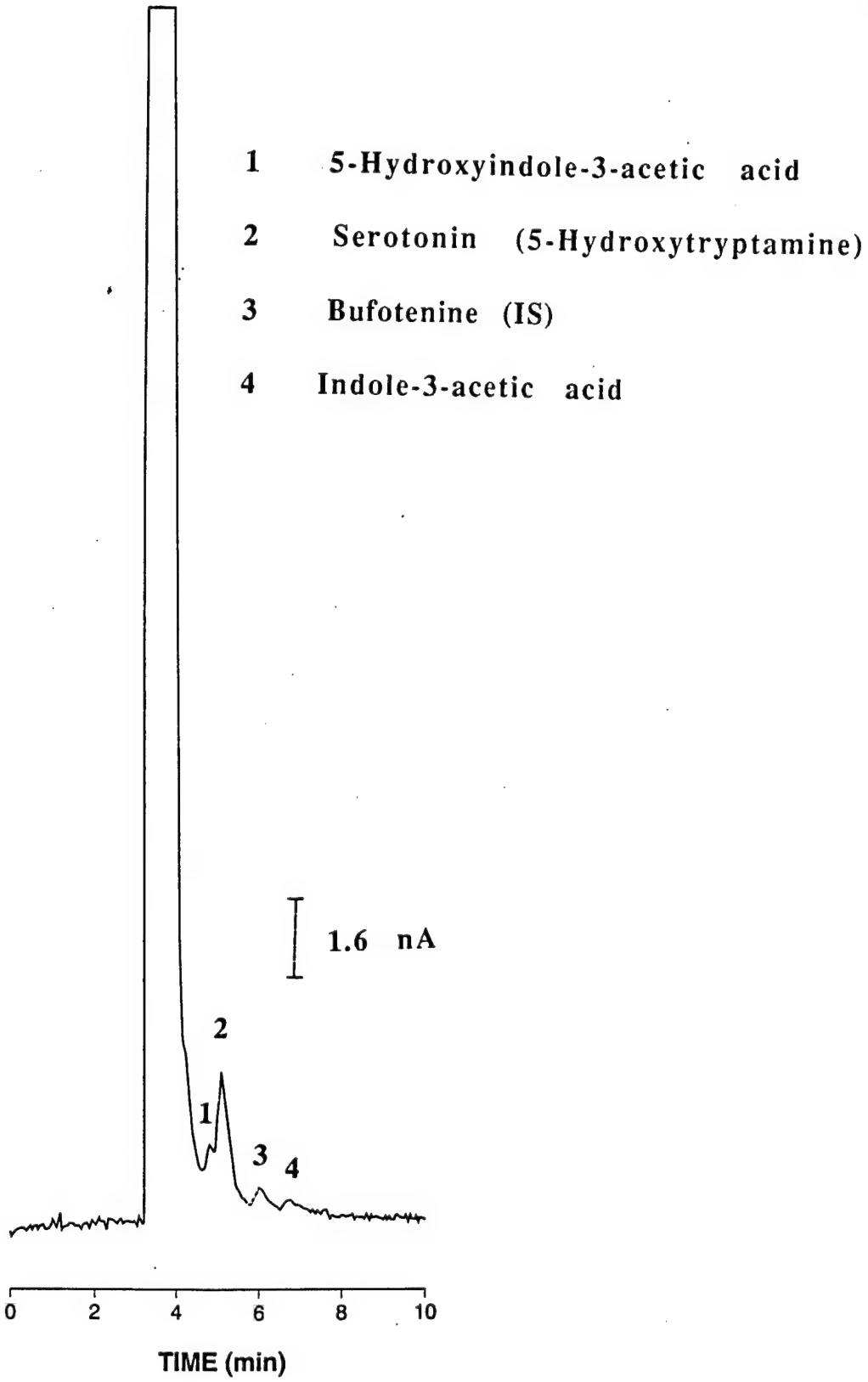



Figure IV.2. Separation of serotonin and metabolites from bufotenine (internal standard).

Table IV.2. Serotonin (5-HT) Levels in SSRI OD's*.

| sample # | Sex | Amount 5-HT | Amount 5HIAA | Amount IAA | Drugs present |
|---|---|---|---|---|---|
| 1 | M | 0.00 | 0.00 | 0.00 | venlafaxine (13.93) *o*-desmethylvenlafaxine (0.25) alcohol (0.22 g/100 ml) nortriptyline (1.60) |
| 2 | F | 22.21 | 0.01 | 0.01 | fluoxetine (1.19) norfluoxetine (0.83) trazodone (32.91) alcohol (0.22 g/100 ml) |
| 3 | F | 0.02 | 0.02 | 0.00 | fluoxetine (1.40) norfluoxetine (0.75) isopropanol (0.24 g/100 ml) acetone (0.13 g/100 ml) |
| 4 | M | 0.02 | 0.00 | 0.11 | fluoxetine (3.67) norfluoxetine (0.38) alcohol (0.03 g/100 ml) |
| 5 | F | 222.22 | 0.00 | 0.78 | fluoxetine (1.73) norfluoxetine (0.93) |

Table IV.2 (Continued).

| sample # | Sex | Amount 5-HT | Amount 5HIAA | Amount IAA | Drugs present |
|---|---|---|---|---|---|
| 6 | F | 1.80 | 0.00 | 0.31 | sertraline (2.52) *n*-desmethylsertraline (4.05) trazodone (0.35) alcohol (0.18 g/100 ml) dextromethorphan (0.49) promethazine (0.98) antipyrine cocaine (<0.05) cocaethylene (<0.05) benzoylecgonine (0.57) |
| 7 | F | 181.43 | 0.02 | 0.12 | sertraline (1.76) *n*-desmethylsertraline (0.15) trazadone (1.41) alcohol (0.19 g/100 ml) propoxyphene (6.60) norpropoxyphene (0.87) codeine (7.00) acetominophen (521.00) ibuprofen (37.00) hydrocodone (1.28) diphenhydramine (1.09) |

Table IV.2 (Continued).

| sample # | Sex | Amount 5-HT | Amount 5HIAA | Amount IAA | Drugs present |
|---|---|---|---|---|---|
| 8 | F | 4.14 | 0.00 | 0.00 | sertraline (1.76) *n*-desmethylsertraline (0.57) trazodone (24.32) diltiazem (1.20) temazepam (7.75) |
| 9 | M | 11.30 | 0.00 | 0.00 | sertraline (1.25) *n*-desmethylsertraline (1.16) cocaine (1.4) benzoylecgonine (3.7) nordiazepam (<0.1) |

* (5-HT concentrations in umol/L, drug concentrations in mg/L).

from two ostensibly healthy adult volunteers was obtained, and PPP immediately prepared from these samples. The samples were spiked with either 5 or 500 ng/mL 5-HT prior to protein precipitation, and the 5-HT content of these samples was repeatedly quantitated over the period of three consecutive days. The samples were stored at 4°C in between analyses. On the first day, samples were spiked immediately, while samples were still warm, to take into consideration any enzymatic changes to 5-HT levels. The levels found in this analysis are reported in Table IV.3. The elevated 5-HT level in the 5 ng/ml sample analyzed on the

Table IV.3. Serotonin (5-HT) & Metabolite Levels in Stability Study (PPP prepared immediately)*.

| Subject | Time Since Sample Taken (Days) | 5-HT | 5HIAA | IAA |
|---|---|---|---|---|
| 1 (5 ng/mL spike) | 0 | 2.14 | 0.00 | 0.01 |
| | 1 | 3.20 | 0.03 | 0.02 |
| | 2 | 27.21 | 6.17 | 0.48 |
| 2 (5 ng/mL spike) | 0 | 1.30 | 0.00 | 0.01 |
| | 1 | 1.94 | 0.00 | 0.01 |
| | 2 | 0.57 | 2.50 | 3.08 |
| 1 (500 ng/mL spike) | 0 | 6.92 | 0.00 | 0.01 |
| | 1 | 7.21 | 0.12 | 0.03 |
| | 2 | 7.41 | 0.13 | 0.49 |
| 2 (500 ng/mL spike) | 0 | 6.41 | 0.00 | 0.02 |
| | 1 | 6.41 | 0.01 | 0.02 |
| | 2 | 4.33 | 0.09 | 0.79 |

* (Concentrations in umol/L).

third day is most likely due to contamination from platelet 5-HT attributable to the fact that anticoagulant was not added directly to the syringes used to draw the blood, rather than due to centrifugation, as a low relative g- force was used. However, the data do suggest that changes in 5-HT concentrations in PPP occur over time.

The effect of prolonged freezing of a sample prior to preparation of PPP was also investigated, as postmortem samples may be stored frozen for prolonged periods prior to 5-HT assay. After the first day, it became impossible to determine whether samples were truly platelet-poor, due to release of hemoglobin from hemolyzed red blood cells. A clear supernate as observed in blood samples

centrifuged immediately after being taken was not observed after centrifuging blood samples stored at 4°C for at least one day. PPP samples prepared on each of two days following the day blood was drawn were spiked with 5 ng/ml of 5-HT after thawing to room temperature. Results of analysis of these samples are listed in Table IV.4. Comparison of these values with those in Table IV.3 reveals marked differences in concentrations of 5-HT and its metabolites between these samples and those PPP samples which were prepared immediately after the blood was taken and then stored. For this reason, it is recommended that PPP be prepared immediately after samples are taken and then frozen for either shipment or later analysis.

Table IV.4. Serotonin (5-HT) Levels in Stability Study (PPP prepared on day of analysis)*.

| Subject | Time Since Sample Taken (Days) | 5-HT | 5HIAA | IAA |
|---|---|---|---|---|
| 1 (5 ng/mL spike) | 1 | 0.26 | 0.01 | 0.00 |
| | 2 | 0.14 | 0.00 | 0.04 |
| 2 (5 ng/mL spike) | 1 | 0.29 | 0.07 | 0.05 |
| | 2 | 0.40 | 0.04 | 0.05 |

* (Concentrations in umol/L).

Where possible, metabolite concentrations were quantitated in these cases in addition to 5-HT. Although 5HIAA and IAA could only be detected in some of the case samples, 5-HT could be detected in all but one of the SSRI overdoses and all of the cases where no drug was present. In the one case where 5-HT could not

be detected, however, neither of the metabolites could be detected, either. This may be due to degradation over time.

All samples from SSRI overdoses had 5-HT levels above those of samples where no drug was present. All but three subjects among the SSRI overdoses where female. In one study on the status of 5-HT in whole blood and plasma (6), the authors found differences between men and women in plasma and whole blood 5-HT (both higher in women) and in plasma 5HIAA (lower in women). They concluded these differences may reflect a differential whole body 5-HT function between the sexes. This may account for some of the variation in PPP 5-HT levels among the overdose cases. However, contamination from platelet 5-HT, either due to excessively high g-forces used in centrifugation or not adding anticoagulant directly to the syringe used to draw blood, was likely a major factor contributing to artificially high concentrations.

The subject in Case 2 had elevated concentrations of trazodone and fluoxetine, which may have contributed to her relatively high 5-HT level, although contribution from platelet 5-HT to plasma levels due to the non-presence of an anticoagulant in the syringe cannot be ruled out. Similarly, Cases 7 and 9 both had sertraline and *n*-desmethyl-sertraline concentrations above those associated with the onset of toxic side effects, as well as significant levels of many other drugs.

It is questionable from these results whether postmortem rupture of platelets occurs, in a manner similar to hemolysis of red

blood cells. There is no information in the literature to help determine this. However, at the beginning of this study, a cursory investigation of relative 5-HT concentrations in whole blood to that in theoretically platelet-poor samples revealed considerably lower levels in whole blood. In *Fundamentals of Clinical Chemistry*, Tietz *et al.* (144) state that in grossly hemolyzed serum, a dilution of the serum component of interest occurs if its concentration in red blood cells is lower than that in plasma. If platelets rupture postmortem, such differences might not be observed. Because this was not the case, it may be that platelets do not rupture at death. However, it is also possible that the sample preparation method in this chapter simply served to separate hemolyzed red blood cells from the ruptured platelets, which would have the effect of increasing 5-HT concentrations measured by this method.

It has been suggested that diminished release of amine combined with depleted stores of 5-HT may indicate a reduction in turnover of this monoamine in the brain, and that the defect may lie somewhere between the transport of 5-HT into the brain and synthesis and storage of 5-HT (41). If this is true, increased serum serotonin levels due to the presence of serotonergic drugs might approach dangerous levels in such subjects due to inability to transport and store sufficient 5-HT into the brain.

One subject among the SSRI overdose cases had cardiovascular disease (Case 5). Her PPP 5-HT and IAA levels were higher than any other sample, although 5HIAA could not be detected.

Assuming minimal contamination from platelet 5-HT, which may not be a reasonable assumption, her elevated IAA level suggests her 5-HT levels may have been higher at the time of death.

**4.4. Conclusions.**

It appears that using a conventional method for determination of 5-HT in postmortem plasma may be feasible, although several questions regarding feasibility of doing so are raised by the results of this experiment which may be difficult to answer. These problems are contamination of plasma 5-HT from platelets made unobservable by postmortem hemolysis, further hemolysis of remaining intact blood cells due to repeated freezing and thawing of blood samples prior to analysis, and instability of plasma 5-HT strictly attributable to removal from intact tissue, such as that which has been observed for brain samples stored at room temperature. The contribution from each of these problems to variability in postmortem plasma 5-HT levels are difficult to isolate, but with more time, experiments could be carried out in an attempt to determine this.

The first problem regarding hemolysis upon death is perhaps the most difficult one to overcome, as SSRI overdoses are often suicides or accidental deaths in which no one witnessed them, and a pathologist is not immediately able to come to the scene to take a blood sample. To maximize the possibility of accurately assaying 5-HT, therefore, a very motivated pathologist would be required to go to the scene of a large number of deaths over a specified period

of time to take a blood sample as soon after death as possible. Even with such vigilance, however, the postmortem interval might be too long in most cases to ensure minimal hemolysis. Additionally, plasma would have to be immediately separated after taking the blood sample, so the pathologist would have to take equipment for centrifuging samples with him or her to the scene. The platelet-poor samples could then be stored at 4°C until analysis, which would have to be performed within 12-24 hours, to minimize any time-related changes in plasma 5-HT content.

To investigate the possibility of postmortem platelet rupture, the following experiment could be performed. Duplicate whole blood and platelet-poor specimens from volunteers would be prepared via the protein precipitation method, in addition to the same two types of specimens from SSRI overdose case samples. For whole blood, two types of samples would be analyzed. The first would be samples subjected to protein precipitation immediately after being taken, in which presumably minimal hemolysis occurs. The second would be whole blood placed in an ultrasonerator to induce hemolysis, then subjected to protein precipitation. This would be done for both volunteer and postmortem specimens. The results would then be compared. Similar results between whole postmortem serum which has not been ultrasonerated and volunteer samples subjected to the same conditions would indicate that platelet rupture does not occur. Similarities between theoretically platelet-poor postmortem serum and corresponding

volunteer specimens (in comparison to the other sample types) would indicate whether the postmortem specimens were truly platelet poor.

The second problem, related to repeated freezing and thawing of samples prior to analysis, could easily be eliminated assuming the experiment was not carried out retrospectively, such that samples would enter the laboratory and immediately undergo protein precipitation and 5-HT assay prior to any other analyses requested. In contrast, the samples discussed in this chapter had all been stored for at least one month prior to being analyzed for 5-HT content. On a related note, blood bank blood is probably not suitable for use as a 5-HT blank due to hemolysis as already discussed. Plasma separated from healthy volunteers not taking serotonergic drugs might be more suitable for purposes of calibration and quantitation.

The last problem, associated with instability of 5-HT in plasma after removal from intact tissue, could be addressed through an experiment similar to the stability study discussed in this chapter. Enough PPP would have to be prepared by the pathologist at the scene for at least three separate analyses. One of these samples would be analyzed as soon as possible after arrival at the laboratory, and the rest stored at 4°C. The 5-HT assay would then be carried out on each of three (or more) consecutive days and the results compared.

Finally, it should be noted that having a larger sample population for both groups studied in this chapter might make it easier to draw conclusions regarding feasibility of using this method on postmortem samples. Based on the results here presented, differences in PPP 5-HT content attributable to SSRI use do appear to be discernible. However, the length of time between autopsy and assay of 5-HT content and contamination from 5-HT in platelets may cause larger differences, and should be addressed prior to application of this method to an operational laboratory setting.

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## APPENDIX: LIST OF ABBREVIATIONS

GC/MS - gas chromatography/mass spectrometry

HPLC - high performance liquid chromatography

PDA - photodiode array detection

NMR - nuclear magnetic resonance

LC-MS - liquid chromatography-mass spectrometry

5-HT - 5-hydroxytryptamine

AC - adenyl cyclase

PLC - phospholipase C

SSRI - selective serotonin reuptake inhibitor

MAO-A - monoamine oxidase-A

5HIAA - 5-hydroxyindole-3-acetic acid

SS - serotonin syndrome

NMS - neuroleptic malignant syndrome

TCA - tricyclic antidepressant

ST - serotonin

DA - dopamine

NE - norepinephrine

musc - muscarinic receptor

$V_d$ - volume of distribution

A - amount of drug in body

C - plasma drug concentration

CYP- cytochrome P-450 isoenzyme

mCPP - *m*-chlorophenylpiperazine

NSAID - nonsteroidal anti-inflammatory drug

T - tramadol

GC/NPD - gas chromatography with nitrogen-phosphorous detection

NDT - *n*-desmethyltramadol

ODT - *o*-desmethyltramadol

MBTFA - *n*-methyl-bis(trifluoroacetamide)

AcN - acetonitrile

$R_s$ - resolution

$t_{R,x}$ - retention time of analyte x

$W_x$ - peak width of analyte x

LOD - limit of detection

LOQ - limit of quantitation

$SD_o$ - standard deviation at concentration = 0

Q.I.D. - quater in die (4 times daily)

Hx - history

Rx - prescription

MAOI - monoamine oxidase inhibitor

$t_{1/2}$ - half-life

IS - internal standard

OTPA - beta-(3-oxo-s-triazolic(4-3α )pyridin-2-yl)propionic acid

Traz - trazodone

neg - negative

Bup - bupropion

TAA - bupropion's threoamino alcohol metabolite

BMM - bupropion morpholinol metabolite

Fluox - fluoxetine

Norflu - norfluoxetine

Sert - sertraline

*n*-dSert - *n*-desmethylsertraline

Par - paroxetine

Ven - venlafaxine

ODV - *o*-desmethylvenlafaxine

PPP - platelet-poor plasma

IAA - indole-3-acetic acid

g - relative centrifugal force

r - spinning radius in centimeters

n - revolutions per minute